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Expression of Cyclin B1 and cdc2 in Nodal Non-Hodgkin's Lymphoma and its Prognostic Implications

To investigate the role of cyclin B1 and cdc2 in the pathogenesis and progression of malignant lymphoma, 68 cases of nodal non-Hodgkin's lymphoma were examined about the expression of cyclin B1 and cdc2 along with p53 and Ki-67 by immunohistochemical method. The correlation of their expression with various clinicopathologic findings was also analyzed. Cyclin B1 and cdc2 were diffusely expressed in 39 cases (57.4%) and 54 cases (79.4%) out of 68 cases studied, respectively. The mean labeling indices of cyclin B1 and cdc2 in malignant lymphoma were 31.9% and 68.0%, respectively. In normal lymphoid tissues, cyclin B1 and cdc2 were expressed predominantly in the germinal center with mean labeling indices of 13.9% and 28.3%, respectively. The correlation between the expression of cyclin B1 and cdc2 was noted (p=0.013). The expression of Ki-67 was correlated with that of cyclin B1 (p=0.023) and marginally correlated with that of cdc2 (p=0.056). The expression of cdc2 and p53 in complete remission group to chemotherapy was lower than that of progressive disease group (p=0.047, p=0.049). In multivariate analysis, the clinical stage alone showed significance on overall survival (p=0.049). In conclusion, cyclin B1 and cdc2 appeared to be involved in the genesis or progression of malignant lymphoma and cdc2 can be a useful marker for response to chemotherapy.

Key Words : *Lymphoma, Malignant; Cyclin B1; cdc2*

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INTRODUCTION

Cell cycle progression is governed by cell cycle regulators such as cyclins and cyclin-dependent kinases (cdks). Dysregulated expression of these cyclins, cdks, or both is involved in uncontrolled cell growth and malignant transformation (1). Individual cyclins act at different phases of the cell cycle by binding and activating corresponding cdks. Under normal conditions, cyclins undergo degradation at the end of each functional phase, whereas the level of cdks remains invariable throughout the cell cycle (2, 3). Cyclin B1, which activates cdc2 and regulates progression of cell cycle through G2 and M phases, is recently focused as a subject to be studied. Newly synthesized cyclin B1 binds to the inactive cdc2 at the beginning of G2 phase forming a cyclin B1/cdc2 complex, which can be activated by phosphorylation. This activated kinase complex then phosphorylates a number of proteins important in regulating the G2 to M transition (4). Overexpression of cyclin B1 and cdc2 has been reported in malignancies of breast (5), colon (6), esophagus (7), liver (8), and so on. According to recent studies, p53 regulates G2 checkpoint through repression of cyclin B1 and cdc2 transcription (9). In this study, the authors examined the expression of cyclin B1 and cdc2 along with p53 and Ki-67 in 68 cases of nodal non-Hodgkin's lymphoma by immunohistochemical method and analyzed the correlation of the expression of cyclin B1 and cdc2 with various clinicopathologic findings including response to chemotherapy andoverall survival.

MATERIALS AND METHODS

Study population

Sixty-eight cases of histologically diagnosed nodal non-Hodgkin's lymphomas from 1987 to 1998 at Hanyang University Medical Center were submitted for this study. Follow-up data of the cases were retrieved from clinical records of the hospital retrospectively. The classification and diagnoses of malignant lymphoma were made based on the new World Health Organization classification of lymphomas (10). The staging of nodal non-Hodgkin's lymphoma was performed according to the Ann Arbor staging system (11).

The patients were grouped as complete remission, partial

remission, progressive disease, and stable disease according to chemotherapy response. As control, 10 cases of reactive hyperplasia of lymph nodes and tonsils were used.

Immunohistochemical staining

Paraffin-embedded, 4 μm-thick tissue sections were selected for immunohistochemistry. Slides were dehydrated, deparaffinated through xylene, and then rehydrated through graded alcohols. To retrieve the antigenicity, the sections were treated with microwaves in 10 mM citrate buffer at pH 6.0 for 30 min. Then the sections were immersed in methanol containing 2% H_2O_2 for 30 min to block the endogenous peroxidase activity and pretreated with normal goat serum to reduce nonspecific reactions. The sections were incubated at 4℃ overnight with each primary antibody. The primary antibodies used were cyclin B1 (Novocastra, Newcastle, U.K., 1:20), cdc2 (Santa Cruz, CA, U.S.A. 1:100), p53 (Novocastra, 1:50), and Ki-67 (Biogenex, San Ramon, CA, U.S.A. 1:50). Immunohistochemical staining was performed using standard streptavidin-biotin complex procedure. 3,3′-diaminobenzidine was used as a chromogen, and Meyer's hematoxylin was used for counterstaining. In case of malignant lymphoma, after three representative areas were photographed under ×200 magnification, the numbers of entire cell and positively stained cell were counted to assess the expression of cyclin B1, cdc2, p53, and Ki-67. On immunostaining for cyclin B1 and cdc2, cytoplasmic and/or nuclear staining were considered positive. Whereas, only nuclear staining was considered positive on immunostaining for p53 and Ki-67. Positive staining in more than 5% of the tumor cells was considered positive. In case of normal lymphoid tissue, three representative areas were photographed under 200 magnificationin germinal center and interfollicular area, respectively.

Statistical analysis

The relationships between expression of cyclin B1, cdc2, p53, Ki-67 and various clinicopathological findings were evaluated using χ^2 test and Kruskal-Wallis test. The Pearson correlation and paired two-tailed Student's t-test were used to examine the associations between the expression of cyclin B1, cdc2, p53, and Ki-67. Kaplan-Meier survival curves were made to assess whether any level of cyclin B1 and cdc2 had any effect on overall survival of patients with malignant lymphoma and the resulting curves were compared using the log-rank test. Cox's proportional hazards regression model was used as multivariate analyses to find significant independent prognostic markers.

A value less than 0.05 was considered to be statistically significant. These statistical analyses were performed with SPSS for Windows (version 7.5).

Table 1. Clinical data of 68 cases with nodal non-Hodgkin's lymphoma

Sex	Male	42
	Female	26
Age (yr)		4-78 (mean, 45.8)
Clinical follow-up duration(months)		1-113 (mean, 23.4)
Response to chemotherapy		
	Complete remission	22
	Partial remission	6
	Stable disease	3
	Progressive disease	11
Ann Arbor stage	I	5
	II	11
	III	12
	IV	26
	Undetermined	14

RESULTS

Clinical data

The clinical data of the patients with malignant lymphoma are summarized in Table 1. Of 68 cases, 42 were men and 26 were women. The age ranged from 4 to 78 yr, with a mean age of 45.8 yr. The follow-up period ranged from 1 month to 113 months. The median follow-up period was 23.4 months. According to the chemotherapeutic response, the patients were grouped as complete remission (n=22), partial remission (n=6), stable disease (n=3), and progressive disease (n=11). There were 5 cases with stage I, 11 cases with stage II, 12 cases with stage III, and 26 cases with stage IV. Stage was not confirmed in the remaining 14 cases due to data loss. The histologic subtypes included diffuse large B-cell lymphoma (DLBL, n=35), follicular lymphoma (FCL, n=5), lymphoblastic lymphoma (LB, n=7), Burkitt's lymphoma (n=6), peripheral T-cell lymphoma (PTCL, n=10), anaplastic large cell lymphoma (ALCL, n=3), angioimmunoblastic T-cell lymphoma (AILD, n=1), and extranodal NK/T cell lymphoma (n=1).

Expression of cyclin B1 and cdc2 in normal lymphoid tissues

The staining pattern of cyclin B1 and cdc2 exhibited definite regional differences in normal lymphoid tissues (Fig. 1). The germinal center was characteristically highlighted. On the other hand, mantle zone and parafollicular T-cell zone were negligible. Cyclin B1 and cdc2 were expressed mainly in the cytoplasm, and sometimes in the nuclei. The labeling indices of cyclin B1 were 13.9%, 0.8%, and 1.2% in germinal center, mantle zone, and parafollicular T-cell zone, respectively. The labeling indices of cdc2 were 28.3%, 1.3%, and 2.1% in germinal center, mantle zone, and parafollicular T-cell zone, respectively. Ki-67 and p53 were detected in the nuclei of germinal center cells. In the mantle and parafollicular T-cell zone, the positive cells were rare.

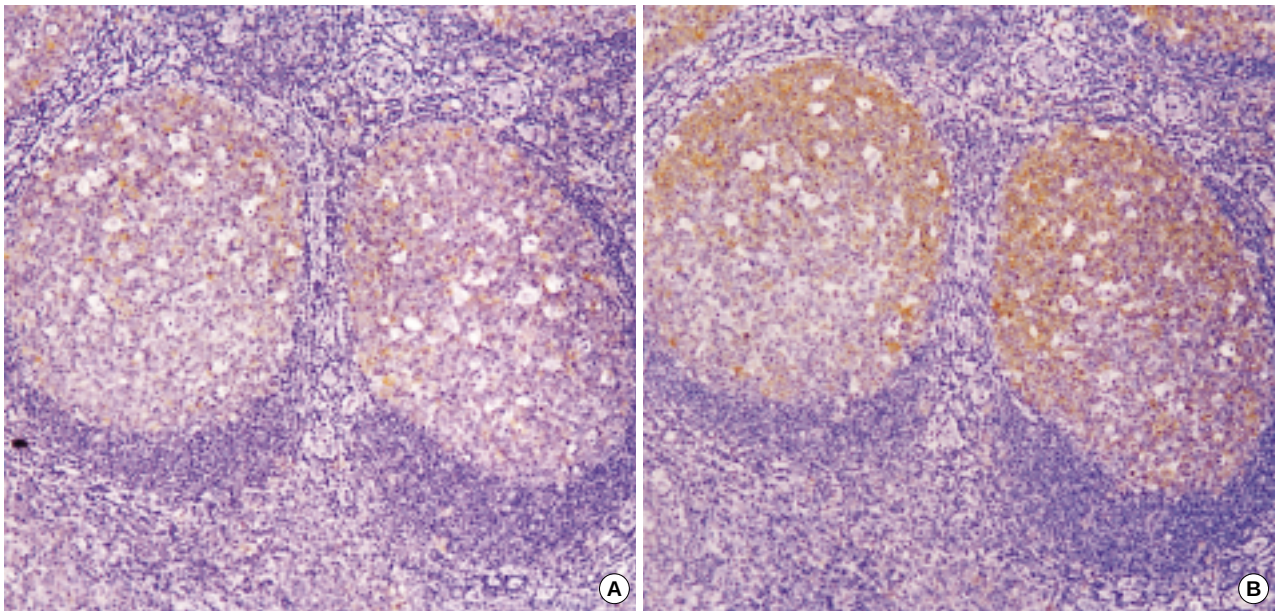

Fig. 1. Immunostating for cyclin B1 and cdc2 in the reactive tonsil (×200). Cyclin B1 (A) and cdc2 (B) are expressed predominantly in germinal center cells. whereas mantle cells and interfollicular small lymphocytes are almost negative.

Table 2. Percentages of cyclin B1, cdc2, and p53 expressions in 68 cases of nodal non-Hodgkin's lymphoma according to histologic types

Type	Cyclin B1 (%)	cdc2 (%)	p53 (%)
DLBL (n=35)	20 (57.1%)	27 (77.1%)	12 (34.3%)
FCL (n=5)	3 (60%)	5 (100%)	1 (20%)
LB (n=7)	4 (57.1%)	6 (85.7%)	0
Burkitt (n=6)	3 (50%)	6 (100%)	2 (33.3%)
PTCL (n=10)	6 (60%)	6 (60%)	4 (40%)
ALCL (n=3)	2 (66.7%)	2 (66.7%)	2 (66.7%)
AILD (n=1)	1 (100%)	1 (100%)	0
Extranodal (n=1)	0	1 (100%)	1 (100%)

DLBL, diffuse large B-cell lymphoma; FCL, follicular lymphoma; LB, lymphoblastic lymphoma; Burkitt, Burkitt's lymphoma; PTCL, peripheral T-cell lymphoma; ALCL, anaplastic large cell lymphoma; AILD, angioimmunoblastic T-cell lymphoma; Extranodal, extranodal NK/T cell lymphoma.

Table 3. Multivariate Cox regression analysis for individual parameters

Factor	Hazard ratio	95% confidence interval	*p* value
Age	2.437	0.997-1,034	0.094
Sex	0.894	0.645-3.333	0.361
Stage	3.850	0.936-1.827	0.049*
Cyclin B1	0.237	0.984-1.025	0.678
Cdc2	0.144	0.989-1.013	0.868
p53	0.358	0.699-1.458	0.959
Ki-67	2.089	0.981-1.023	0.870

*: Statistically significant. Cox proportional hazards regression model is as follows: $h_1(t)=h_0(t)\exp(\beta_1$ age group$+\beta_2$ sex$+\beta_3$ stage group$+\beta_4$ cyclin B1$+\beta_5$ cdc2$+\beta_6$ p53$+\beta_7$ Ki-67). Regression analysis was performed using forward stepwise method. Age: 0=<60, 1=>60; Sex: 0=male, 1=female; Stage group: 0=stage I/II, 1=stage III/IV; Cyclin B1: 0=negative, 1=positive; Cdc2: 0=negative, 1=positive; p53: 0=negative, 1=positive; Ki-67: 0=negative, 1= positive.

Expression of cyclin B1, cdc2, p53, and Ki-67 in nodal non-Hodgki's lymphoma

Cyclin B1 and cdc2 were diffusely expressed in 39 cases (57.4%) and 54 cases (79.4%) of 68 cases with malignant lymphomas, respectively (Fig. 2A, B). The mean labeling indices of cyclin B1 and cdc2 were 31.9% and 68.0%, respectively. The intracellular localization of immunostaining in malignant lymphomas were not different from those of normal lymphoid tissues. The immunostaining of cyclin B1 and cdc2 was detected predominantly in the cytoplasm. There was no statistically significant difference in cyclin B1 and cdc2 expression according to histologic types (Table 2). Cyclin B1 and cdc2 showed no correlation with age, sex, and stage. Cdc2 showed lower expression in complete remission group compared to the expression in progressive disease group, which was statistically significant (p=0.047) (Fig. 3).

p53 was expressed diffusely in 22 cases (32.4%) (Fig. 2C). Ki-67 was expressed diffusely in all cases, with a mean proliferative index of 35.7% (Fig. 2D). Like normal lymphoid tissues, the nuclei showed positive immunostaining against p53 and Ki-67. p53 showed high expressions in anaplastic large cell lymphoma and angiocentric lymphoma, but the difference was not statistically significant (Table 2). p53 and Ki-67 showed no correlation with age, sex, and stage. p53 showed a lower expression in complete remission group than

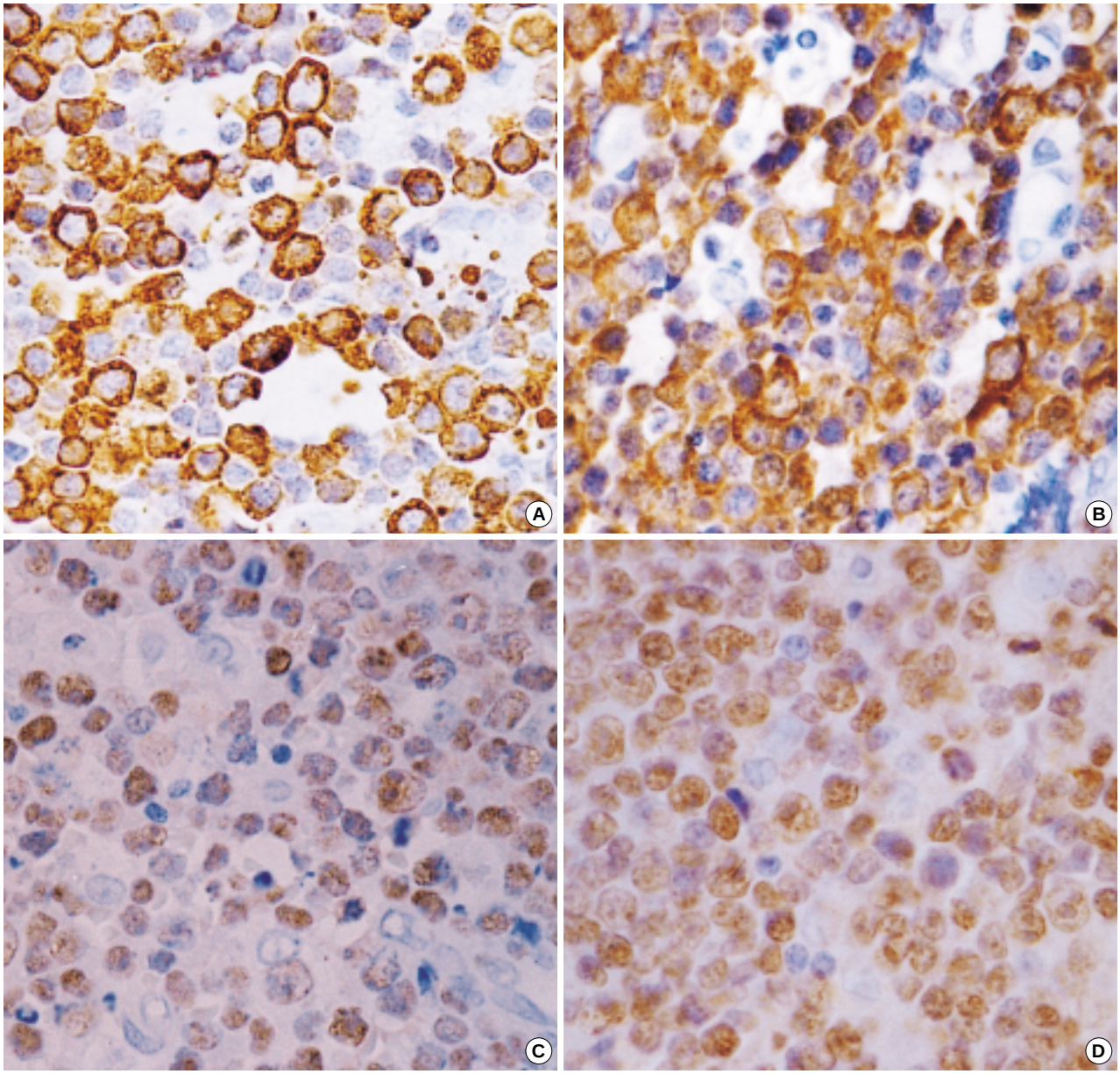

Fig. 2. Immunostaining for cyclin B1 (A), cdc2 (B), p53 (C), and Ki-67 (D) in the case of diffuse large B-cell lymphoma (×400). Cyclin B1 (A) and cdc2 (B) are diffusely expressed in the cytoplasm of lymphoma cells, whereas p53 (C) Ki-67 (D) are diffusely expressed in the nuclei of lymphoma cells.

in progressive disease group, which was statistically significant (p=0.049).

Correlations between cyclin B1, cdc2, p53, and Ki-67 immunostaining in nodal non-Hodgkin's lymphoma

The expression of cyclin B1 had a tendency to increase according to that of cdc 2, which was statistically significant (p=0.013). Cyclin B1 showed a significant correlation with Ki-67 (p=0.023). Cdc2 and Ki-67 showed a marginally significant correlation (p=0.056). However, p53 was not correlated with cyclin B1, cdc2, or Ki-67.

Survival analysis

Survival analysis was performed in 60 patients with a mean observation duration of 23.4 months. Cyclin B1, cdc2, and p53 showed no significant difference in survival regardless of the cut-off value in the Kaplan-Meier survival curves (Fig. 4). On multivariate analysis, the stage alone was a significant variable affecting overall survival (Table 3). Cyclin B1, cdc2, and p53 had no influence on the overall survival.

DISCUSSION

To evaluate the role of cyclin B1/cdc2 in the pathogenesis of malignant lymphoma and to determine whether the overexpression of cyclin B1/cdc2 influences the prognosis including response to chemotherapy and overall survival, the authors studied the aberrant expression of cyclin B1 and cdc2 in 68 patients with nodal non-Hodgkin's lymphomas.

In this immunohistochemical study, the mean labeling indices of cyclin B1 and cdc2 in malignant lymphomas were 31.9% and 68.0%, respectively. The mean labeling indices of cyclin B1 and cdc2 in the germinal center of normal lymphoid tissues were 13.9% and 28.3%, respectively, which are much lower than those of malignant lymphomas. The staining patterns between malignant lymphomas and reactive lymphoid tissues were quite different. In reactive lymphoid tissues, the staining pattern showed a regional difference, that is, only germinal centers were highlighted. In contrast, cyclin B1 and cdc2 were expressed diffusely in malignant lymphomas. These results suggest that cyclin B1 and cdc2 may play a role in the genesis or progression of malignant lymphoma. How cyclin B1 and cdc2 participate in tumor progression remains unknown. Although it has been revealed that p53 plays a role in arrest at G1 checkpoint through p53-mediated synthesis of the cell cycle inhibitor, p21, recent studies revealed that p53 regulates G2 checkpoint in the cell cycle by inactivating cdc2 kinase (9, 12). This inactivation of cdc2 kinase results, at least in part, from repression of cyclin B1 and cdc2 transcription. However, enforced expression of both cyclin B1 and cdc2 leads to an override of p53-mediated G2-M arrest. Because p53 mutation has been found in a variety of malignancies (13, 14), p53 dysregulation may result in a failure of repression of cyclin B1 and cdc2, which may cause overexpression of cyclin B1/cdc2 and G2-M transition without G2 checkpoint or without genomic integrity. On the other hand, because constitutive activation of cyclin B1-associated cdc2 kinase overrides p53-mediated cell cycle arrest, cell cycle can continue without G2 checkpoint in case cyclin B1 is overexpressed. Cyclin B1 overexpression may be caused by impaired proteolytic degradation, unlimited protein synthesis, or by some other reasons. It remains to be elucidated how the overexpression of cyclin B1 and cdc2 is involved in onco-

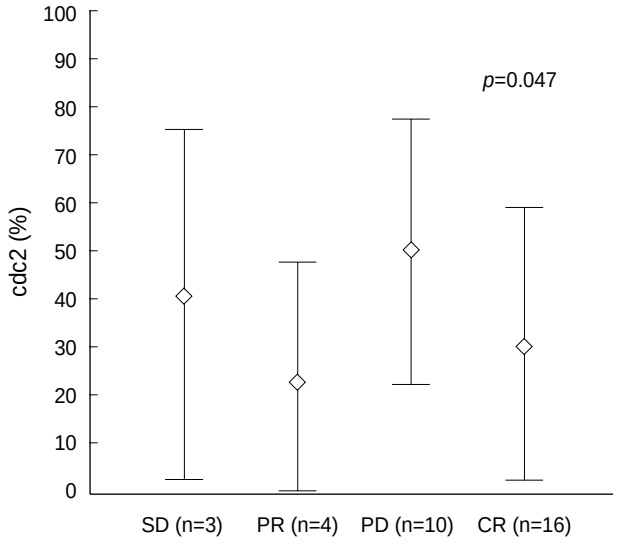

Fig. 3. Results of cdc2 expression according to chemotherapy response. When the complete remission group is compared to the progressive disease group, cdc2 shows lower expression in the former group with a statistical significance (p=0.047).
SD: stable disease, PR: partial remission, PD: Progressive disease, CR: Complete remission.

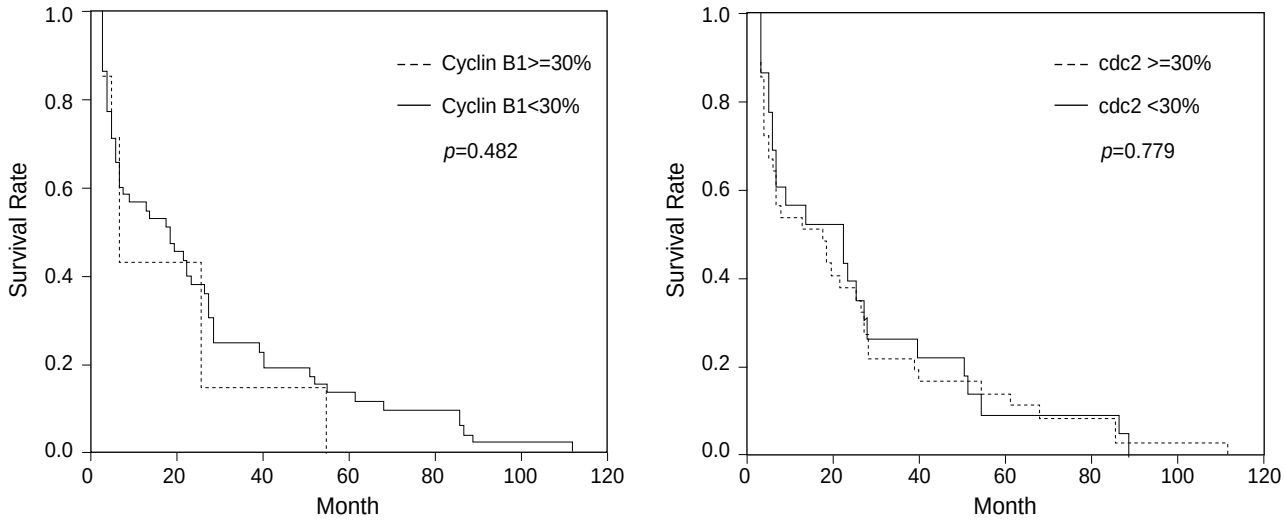

Fig. 4. Kaplan-Meier survival curve stratified according to the extent of cyclin B1 (left) and cdc2 (right) expressions. When the expression of cyclin B1 is stratified as 30% or more (n=9) and below 30% (n=30), the survival curve of cyclin B1 shows no significance (left). When the expression of cdc2 is stratified as 30% or more (n=41) and below 30% (n=13), the survival curve of cdc2 shows no significance.

genesis and tumor progression.

Although there was no significant correlation between the expression of p53 and cyclin B1/cdc2 in this study, more studies are needed to assess the relation between p53 and cyclin B1/cdc2 in malignant lymphoma. Cyclin B1 is normally present only in the later cell cycle phases and re-synthesized as late as the beginning of the S phase, whereas cdc2 is expressed during all phases of the cell cycle except G0 phase (4). This fact can be the reason why the labeling index of cdc2 is higher than that of cyclin B1 in both reactive lymphoid tissue and malignant lymphoma.

Although overexpression of cyclin B1 has been shown to be an important factor affecting survival in several malignant diseases including esophageal squamous cell carcinoma (7), non-small cell lung cancer (15), and hepatocellular carcinoma (8), cyclin B1 and cdc2 showed no influence on survival according to Kaplan-Meier survival curve analysis, regardless of the cut-off value in our study. Moreover, the stage was the only significant factor affecting the overall survival. However, cdc2 showed a lower expression in complete remission group than in progressive disease group, which was statistically significant (p=0.047). Although this fact suggests that cdc2 may be a useful predictor for outcome of chemotherapeutic intervention in malignant lymphoma, the standard percentage of cdc2, which predicts chemotherapy response in non-Hodgkin's lymphoma, should be determined through prospective studies with large numbers of patient group for clinical application.

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