ANTICANCER RESEARCH *24*: 259-264 (2004)

Polo-like Kinase 1 (PLK1) Expression is Associated with Cell Proliferative Activity and Cdc2 Expression in Malignant Lymphoma of the Thyroid

YASUHIRO ITO[1], HIROSHI YOSHIDA[1], FUMIO MATSUZUKA[1], NARIAKI MATSUURA[2], YASUSHI NAKAMURA[3], HIROKAZU NAKAMINE[4], KENNICHI KAKUDO[3], KANJI KUMA[1] and AKIRA MIYAUCHI[1]

[1]*Department of Surgery, Kuma Hospital, 8-2-35, Shimoyamate-dori, Chuo-ku, Kobe 650-0011;*
[2]*Department of Pathology, School of Allied Health Science, Osaka University Faculty of Medicine, 1-7, Yamadaoka, Suita, Osaka 565-0871;*
[3]*Department of Pathology and* [4]*Laboratory Medicine, Wakayama Medical School, Wakayama, Japan*

Abstract. *Background: Polo-like kinase 1 (PLK1) is one of the serine threonine kinases that plays a role in cellular proliferation by activating the CDC25C-CDK1 (cdc2) loop. We recently demonstrated that cdc2 expression is associated with the biological aggressiveness of malignant lymphoma of the thyroid. In this study, we investigated PLK1 expression in thyroid lymphoma in order to elucidate its physiological significance in this disease. Materials and Methods: We immunohistochemically investigated PLK1 expression in 46 cases of thyroid lymphoma and 10 cases of chronic thyroiditis. Results: Normal follicular cells did not express PLK1, whereas follicular cells in those in chronic thyroiditis and malignant lymphoma demonstrated a high level of PLK1 expression. In lymphocytes in chronic thyroiditis with strong lymphocyte infiltration, PLK1 was weakly expressed. In malignant lymphoma, PLK1 expression was observed in all cases, but PLK1 was more strongly expressed in cases with high cell proliferative activity and with cdc2 expression. Conclusion: These results suggest that PLK1 regulates the cell cycle progression of thyroid lymphoma cells in the G2-M-phase.*

Primary malignant lymphoma of the thyroid is an uncommon disease arising from chronic thyroiditis (Hashimoto disease). It generally is a non-Hodgkin's lymphoma (NHL), accounting for less than 5% of malignant tumors of the thyroid (1). Most tumors of thyroid lymphoma grow rapidly causing stenosis of the trachea and even asphyxia. Thus, urgent and accurate diagnosis is required for appropriate treatment, which consists of a combination of chemotherapy (CHOP) and radiation (2). Matsuzuka *et al.* demonstrated that 90% of patients were diagnosed with or suspected of having thyroid lymphoma based on fine-needle aspiration biopsy, although surgical biopsy is required to confirm the histological diagnosis (2). Furthermore, genetic analysis investigating rearrangement of the *IgH* gene can also contribute to establishing a diagnosis (3). Histologically, most thyroid lymphomas are classified as either marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MZBL) or diffuse large B-cell lymphoma (DLBL). Generally, they have a favourable prognosis, but DLBL has a significant potential for worse outcome and is regarded as a high grade malignancy (4).

During neoplasm development, the activity of cell cycle progression is essential. In thyroid lymphoma, we previously demonstrated that G2-M modulators such as cdc2 (cdk1) and cyclin B1 are significantly linked to cell proliferating activity as well as to the degree of malignancy (5). Recently, polo-like kinase 1 (PLK1), a serine threonine kinase belonging to the polo-like kinases (PLKs) family homologous to the *Drosophila polo* kinase, has been identified as a regulator of cell cycle progression (6, 7). Its expression level is low in the G1-phase, elevates in the S-phase and reaches a maximal level in the G2-M-phase. It contributes to cell mitosis by activating the CDC25C-CDK1 amplification loop (6-8). The function of PLK1 in carcinoma has been studied using carcinoma cell lines and tissues and it has been reported that PLK1 is useful for carcinoma diagnosis and may be a target for cancer chemotherapy (9, 10). However, PLK1 expression using a large number of human carcinoma specimens has not been studied in depth, indicating that our knowledge of the physiological role of PLK1 in human carcinoma remains poor.

Correspondence to: Yasuhiro Ito, M.D., Ph.D., Department of Surgery, Kuma Hospital, 8-2-35, Shimoyamate-dori, Chuo-ku, Kobe 650-0011, Japan. Tel: 81-78-371-3721, Fax: 81-78-371-3645, e-mail: ito01@kuma-h.or.jp

Key Words: PLK1, thyroid malignant lymphoma, cell cycle, immunohistochemistry.

In this study, we investigated PLK1 expression in thyroid lymphoma to elucidate whether this kinase plays a role in the progression of this neoplasm.

Materials and Methods

Tissue specimens. Tissue specimens of malignant lymphoma of the thyroid were obtained from 46 patients who underwent surgical biopsy or surgery in the Department of Surgery, Kuma Hospital, Japan between 2000 and 2002. This study was approved by the ethics committee of the hospital and informed consent was obtained from the participating patients. The cases consisted of 22 MZBLs, which were classified as low grade malignancies and 25 DLBLs, which were classified as high grade malignancies. Their histology was diagnosed by investigating their H-E sections and immunohistochemical phenotypes such as the expression of CD10, CD20, CD45RO, CD5, bcl-2, Î -light chain and λ-light chain (11) These pathological classifications were performed according to the WHO classification by two pathologists (Y.N. and H.N.), with expertise in malignant lymphoma. Furthermore, we prepared tissue specimens of chronic thyroiditis obtained from 10 patients who underwent surgery between 2001 and 2002. For immunohistochemical study, tissue specimens were fixed with 10% formalin and paraffin-embedded.

Antibodies. The following antibodies were employed for immunohistochemistry, with their concentrations in parentheses. Monoclonal antibodies against PLK1 (1:100), cdc2 (clone 17, 1:50), p53 (DO7, 1:100) and Ki-67 (MIB-1, 1:50) were purchased from Transduction Laboratories (Lexington, KY, USA), Santa Cruz Biotechnology (Santa Cruz CA, USA), Novocastra (Newcastle, UK) and Ylem (Rome, Italy), respectively. A polyclonal antibody against cyclin B1 (H-433, 1:50) was purchased from Santa Cruz Biotechnology.

Immunohistochemistry. Tissue sections 4 μm thick were dewaxed and endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 15 min. For antigen retrieval, sections were immersed in 0.03 mol/L citrate buffer (pH 6.0) and incubated at 95˚C for 40 min. After rinsing in phosphate-buffered saline pH 7.2 (PBS), 10% bovine serum (Wako, Osaka, Japan) was applied for 20 min to block nonspecific reactions. Sections were then incubated with primary antibody at 4˚C overnight. After rinsing in PBS, specimens were treated with peroxidase-labeled anti-mouse and anti-rabbit immunoglobulins (Nichirei, Tokyo, Japan) for 30 min. The peroxidase reaction was visualized by incubating the sections with 0.02% 3,3'-diaminobenzidine tetrahydrochloride in 0.05 M Tris buffer with 0.01% hydrogen peroxide (Nichirei). The sections were counterstained with hematoxylin. Sections for the negative control were prepared using mouse and rabbit immunoglobulins instead of the primary antibody.

Immunohistochemical evaluation. PLK1 immunoreactivity was observed predominantly in the cytoplasm. We classified the staining intensity of PLK1 into three categories, weak, moderate, and strong, and we regarded cells positive for PLK1 only if strong expression was observed. PLK expression in each case was evaluated based on the positive tumor cell ratio. Findings were then categorized into four groups based on the positive tumor cell rate; (none), less than 5%, (weak), 6-30%, (moderate), 31-59% and (strong), more than 60%. This categorization is fundamentally similar to that in a previous immunohistochemical study using the same antibody (12).

The evaluation of immunohistochemical results for other proteins was the same as that of the previous study (5). Briefly, when more than 50% and 25% of lymphoma cells expressed cdc2 and cyclin B1, respectively, we regarded the cases as positive for these proteins. We regarded cases as aberrantly expressing p53 when more than 10% of lymphoma cells were positive. Furthermore, because Ki-67 LI averaged 34.7±19.5 (mean±SD), we adopted 35% as a cut-off value for statistical analysis.

Statistical analyses. The Chi-square test was performed to examine the relationship between variables. A p value of less than 0.05 was considered significant.

Results

Normal follicular cells did not express PLK1, whereas PLK1 was heterogeneously or diffusely expressed in those forming small follicles typical of chronic thyroiditis and in those in the scattered follicles present in malignant lymphoma (Figure 1a).

Of the 10 cases of chronic thyroiditis, 4 cases showed strong infiltration of lymphocytes, in which PLK1 immunoreactivity was weakly seen (Figure 1a), whereas the remaining 6 cases, which showed only mild lymphocyte infiltration, were classified as negative for PLK1.

PLK1 immunoreactivity was observed in lymphoma cells in all of the 46 cases of malignant lymphoma examined. Thirteen cases (28.3%) were classified as weak, 22 cases (47.8%) as moderate and 11 cases (23.9%) as showing strong expression of PLK1 (Figure 1b, c). We then compared PLK1 expression and clinicopathological parameters of malignant lymphoma (Table I). PLK1 expression was directly linked to the cell proliferating activity of malignant lymphoma evaluated by Ki-67 LI ($p=0.0011$), although it was not related to histology or aberrant p53 expression. Furthermore, PLK1 expression was directly related to cdc2 expression ($p=0.0376$), although it was not related to cyclin B1 expression.

Discussion

In this study, we demonstrated that: 1) follicular cells in small follicles typical of chronic thyroiditis and malignant lymphoma highly expressed PLK1, whereas normal follicular cells only occasionally expressed PLK1; 2) PLK1 expression was observed in all cases of malignant lymphoma; and 3) the PLK1 expression level was directly linked to the cell proliferating activity and cdc2 expression in malignant lymphoma.

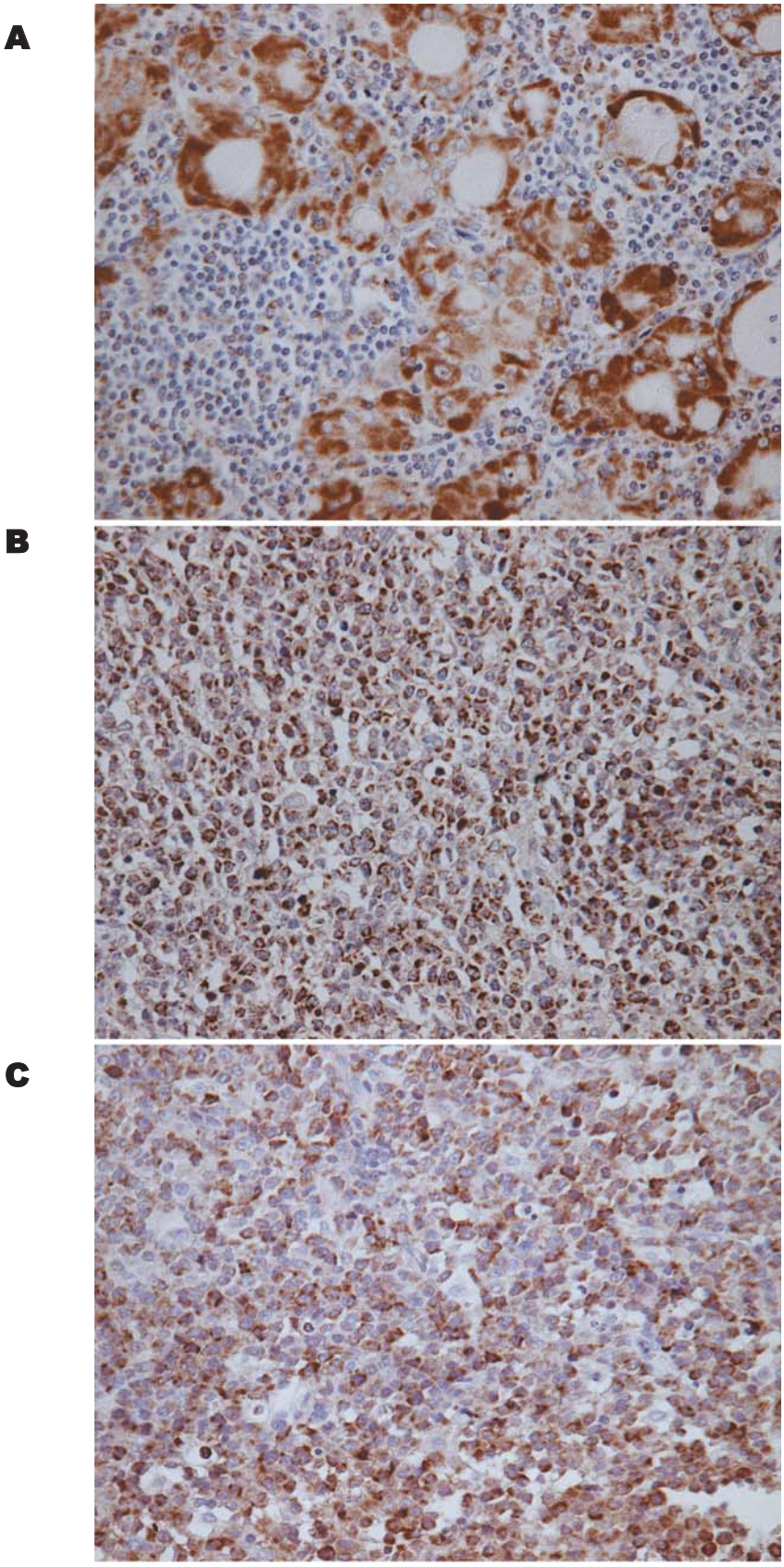

Figure 1. *A. Diffuse expression of PLK1 in follicular cells in chronic thyroiditis. Weak expression of PLK 1 also can be seen in infiltrating lymphocytes. B. Strong expression of PLK1 in lymphoma cells in MZBL. C. Strong expression of PLK1 in lymphoma cells in DLBL. Original magnification; x750*

Table I. *PLK1 expression in malignant lymphoma of the thyroid*

	Weak	Moderate	Strong	Total
Ki-67 labeling index				
≥ 35%	2	10	10	22
< 35%	11	12	1	24
			$p=0.0011$	
Grade of malignancy				
DLBL	5	11	8	24
MZBL	8	11	3	22
			$p=0.2365$	
Aberrant p53 expression				
Yes	4	6	5	15
No	9	16	6	31
			$p=0.5681$	
Cdc2 expression				
High	4	10	9	23
Low	9	12	2	23
			$p=0.0376$	
Cyclin B1 expression				
High	3	9	6	18
Low	10	13	5	28
			$p=0.2818$	
Total	13	22	11	46

To date, PLK1 expression has been studied at the molecular level in some types of carcinoma such as non-small lung carcinoma, squamous cell carcinoma of the head and neck, endometrial carcinoma, gastric carcinoma and esophageal carcinoma (12-16). These studies indicated that the PLK level in these carcinomas, apart from gastric carcinoma, was directly linked to the biological aggressiveness of the tumor. From an immunohistochemical approach, an elevated expression of PLK1 has been reported in only a few carcinomas. In endometrial carcinoma and ovarian carcinoma, PLK1 expression was directly related to histological grade, but not linked to cell proliferating activity or cyclin B1 expression (15, 17). However, Takahashi *et al.* demonstrated that in colorectal carcinoma, PLK1 expression was linked not only to a few important clinical characteristics such as Dukes' classification, tumor invasion, lymph node metastasis and aberrant p53 expression, but also to cell proliferating activity evaluated by Proliferating Cell Nuclear Antigen (PCNA) LI, indicating that PLK1 acts as a stimulator of cell proliferation (12). Our study shows results similar to those of Takahashi *et al.* PLK1 expression was directly related to Ki-67 LI, independently of histological type. Furthermore, although it was not linked to cyclin B1 expression, it was directly related to cdc2 expression, which acts as a regulator of the G2-M-phase by making a complex with cyclin B1. All these results indicate that, in thyroid lymphoma, PLK1 plays a role in stimulating cell cycle progression in the G2-M-phase, as demonstrated in previous *in vitro* studies.

Another interesting finding is that PLK1 expression was observed in follicular cells forming small follicles in chronic thyroiditis and malignant lymphoma. Although the follicular cells were only occasionally positive for Ki-67, PLK1 expression was often positive in these cells. It is therefore suggested that PLK1 plays a role in the regeneration of follicles by responding to the attack of lymphocytes or lymphoma cells.

In conclusion, we demonstrated that PLK1 is associated with cell proliferating activity and cdc2 expression in thyroid lymphoma, indicating that PLK1 may regulate the cell cycle progression of thyroid lymphoma cells in the G2-M-phase. Further studies are necessary to determine whether PLK1 is available as a target of chemotherapy or gene therapy of this disease.

References

1 Schlumberger MJ and Caillou B: Miscellaneous tumors of the thyroid. *In*: Thyroid, Lippincott Williams & Wilkins 8th Ed. p944-948, 2001.

2 Matsuzuka F, Miyauchi A, Katayama S, Narabayashi I, Ikeda H, Kuma K and Sugawara M: Clinical aspects of primary thyroid lymphoma: diagnosis and treatment based on our experience of 119 cases. Thyroid *3*: 93-99, 1993.

3 Matsuzuka F, Fukata S, Kuma K, Miyauchi A, Kakudo K and Sugawara M: Gene rearrangement of immunoglobulin as a marker of thyroid lymphoma. World J Surg *22*: 558-561, 1998.

4 Derringer GA, Thompson LDR, Frommelt A, Bijwaard KE, Heffess CS and Abbondanzo SL: Malignant lymphoma of the thyroid gland. A clinicopathologic study of 108 cases. Am J Surg Pathol *24*: 623-639, 2000.

5 Ito Y, Yoshida H, Matsuzuka F, Matsuura N, Nakamura Y, Nakamine H, Kakudo K, Kuma K and Miyauchi A: Cdc2 expression in primary thyroid lymphoma: its relationship with biological aggressiveness and G2 cyclins. Pathol Res Pract *199*: 533-538, 2003.

6 Hamanaka R, Maloid S, Smith MR, O'Connell CD, Longo DL and Ferris DK: Cloning and characterization of human and murine homologues of the *Drosophila polo* serine-threonine kinase. Cell Growth Diff *5*: 249-257, 1994.

7 Golsteyn RM, Schultz SJ, Bartek J, Ziemiecki A, Ried T and Nigg EA: Cell cycle analysis and chromosomal localization of human PLK1, a putative homologue of the mitotic kinases *Drosophila polo* and *Saccharomyces cerevisiae* Cdc5. J Cell Sci *107*: 1509-1517, 1994.

8 Golsteyn RM, Schulttz SJ, Bartek J, Ziemiecki A, Ried T and Nigg EA: Cell cycle regulation of the activity and subcellular localization of PLK1, a human protein kinase implicated in mitotic spindle function. J Cell Biol *129*: 1617-1628, 1995.

9 Holtrich U, Wolf G, Brauninger A, Karn T, Bohme B, Rubsamen-Waigmann H and Strebhardt K: Induction and down-regulation of PLK, a human serine/threonine kinase expressed in proliferating cells and tumors. Proc Natl Acad Sci USA *91*: 1736-1740, 1994.

10 Yuan J, Horlin A, Hock B, Stutte HJ, Rubsamen-Waigmann H and Strebhardt K: Polo-like kinase, a novel marker for cellular proliferation. Am J Pathol *150*: 1165-1172, 1997.
11 Ito Y, Yoshida H, Matsuzuka F, Matsuura N, Nakamura Y, Nakamine H, Kakudo K, Kuma K and Miyauchi A: Expression of the components of the Cip/Kip family in malignant lymphoma of the thyroid. Pathobiology, in press.
12 Takahashi T, Sano B, Nagata T, Kato H, Sugiyama Y, Kunieda K, Kimura M, Okano Y and Saji S: Polo-like kinase 1(PLK1) is overexpressed in primary colorectal cancers. Cancer Sci *94*: 148-152, 2003.
13 Wolf G, Elez R, Doermer A, Holtrich U, Ackermann H, Stutte HJ, Altmannsberger HM, Rubsamen-Waigmann H and Strebhardt K: Prognostic significance of polo-like kinase (PLK) expression in non-small cell lung cancer. Oncogene *14*: 543-549, 1997.
14 Knecht R, Elez R, Oechler M, Solbach C, von Ilberg C and Strebhardt K: Prognostic significant of polo-like kinase (PLK) expression in squamous cell carcinomas of the head and neck. Cancer Res *59*: 2794-2797, 1999.
15 Takai N, Miyazaki T, Fujisawa K, Nasu K, Hamanaka R and Miyakawa I: Polo-like kinase (PLK) expression in endometrial carcinoma. Cancer Lett *169*: 41-49, 2001.
16 Tokumitsu Y, Mori M, Tanaka S, Akazawa K, Nakano S and Niho Y: Prognostic significance of polo-like kinase expression in esophageal carcinoma. Int J Oncol *15*: 687-692, 1999.
17 Takai N, Miyazaki T, Fujisawa K, Nasu K, Hamanaka R and Miyakawa I: Expression of polo-like kinase in ovarian cancer is associated with histological grade and clinical stage. Cancer Lett *164*: 41-49, 2001.

Received September 16, 2003
Revised November 10, 2003
Accepted December 2, 2003