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Immunohistochemical Analysis of Cyclin D1 Protein in Hematopoietic Neoplasms with Special Reference to Mantle Cell Lymphoma

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Immunohistochemical expression of PRAD1/cyclin D1 protein has been investigated in 106 tissue specimens of 104 cases of lymphoma, non-neoplastic lymphoid disorders and other hematologic malignancies by employing the monoclonal antibody 5D4 with formalin-fixed paraffin-embedded sections, using the microwave oven heating method. Positive neoplastic cells were found in 60 (74%) of 81 cases of non-Hodgkin's lymphoma. The positivity pattern was nuclear in 17 (85%) of 20 cases of mantle cell lymphoma in which cytoplasmic staining was also seen. This pattern of cyclin D1 positivity was in contrast to the negative staining of normal reactive mantle zones. In the other cases, positivity appeared to lie within the cell cytoplasm without nuclear staining, and most of the nodal follicular and diffuse B-cell lymphomas variously expressed PRAD1/cyclin D1. In contrast, the reaction was absent in a significant number of T-cell and extranodal B-cell lymphomas. Immunolocalization of PRAD1/cyclin D1 expression appears to be a useful diagnostic adjunct to discriminate mantle cell lymphoma from other non-Hodgkin's lymphomas.

Key words: Malignant lymphoma — PRAD1 — BCL-1 — Cyclin D1 protein — Immunohistochemistry

Mantle cell lymphoma is a nosological term proposed by Banks *et al.*[1] It is considered to be a neoplastic equivalent of the CD5-positive subpopulation of cells in the mantle zone, based on immunologic and enzyme cytochemical characteristics, and is a distinct clinicopathological entity as previously indicated by several authors.[2,3] This lymphoma is equivalent to lymphocytic lymphoma of intermediate differentiation in the modified Rappaport scheme,[4,5] diffuse centrocytic lymphoma in the Kiel classification,[6] and mantle zone lymphoma, a term popularized by Weisenburger *et al.*[7]

Recent studies have shed light on the pathogenesis of mantle cell lymphomas. They are often associated with a characteristic translocation, t(11;14)(q13;q32),[8] involving a rearrangement of the *BCL-1* locus.[9,10] Although the breakpoints in the *BCL-1* locus are not tightly clustered, Williams and colleagues[11] found rearrangements of *BCL-1* in 73% of their cases of mantle cell lymphoma using a panel of four probes, though such rearrangements were rarely found in other B-cell lymphomas. Recently, an oncogene, *PRAD1*/cyclin D1 gene,[12–14] has been identified to be the transcription unit within the *BCL-1* locus by gene walking,[15] and has been shown to be overexpressed in mantle cell lymphoma.[16–20] We have reported PRAD1/cyclin D1 overexpression in t(11;14)(q13;q32) cell lines and also in a case with a variant *BCL-1* translocation, t(11;22)(q13;q11).[17,18] The latter case was further demonstrated to have the break-point at exon 5 of the *PRAD1* gene.[19] These data indicate the identity of *PRAD1*/cyclin D1 and *BCL-1* genes. Thus, rearrangement of *BCL-1* resulting in the increased expression of a novel cyclin, D1, appears to be a specific and important step in the development of lymphomas of this group. Deregulated PRAD1/cyclin D1 expression may drive uncontrolled mitotic cycling of 11q13 translocation-carrying cells. It is questionable, however, whether significant PRAD1/cyclin D1 protein is present only in the lymphomas with chromosomal rearrangement that deregulates the *BCL-1/PRAD1* gene.

To elucidate the role of the protein product of the *BCL-1/PRAD1* gene in lymphoproliferative disorders, a

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monoclonal antibody was raised against a recombinant PRAD1/cyclin D1 product and was shown to recognize the product at a level corresponding to that of its mRNA expression in human cells.[21] In the current immunohistologic study, we employed this antibody to detect the PRAD1/cyclin D1 protein in paraffin sections of normal and malignant lymphoid tissues. PRAD1/cyclin D1 protein was detected as characteristic nuclear staining in the majority of cases of mantle cell lymphoma, and these neoplastic cells clearly differ from their nonmalignant counterpart (mantle zone cells), in which PRAD1 protein is undetectable. We also sought to contrast the architectural distribution of PRAD1/cyclin D1 protein-positive cells in reactive lymphoid processes versus malignancy to assess the diagnostic value of PRAD1/cyclin D1 immunohistochemistry.

MATERIALS AND METHODS

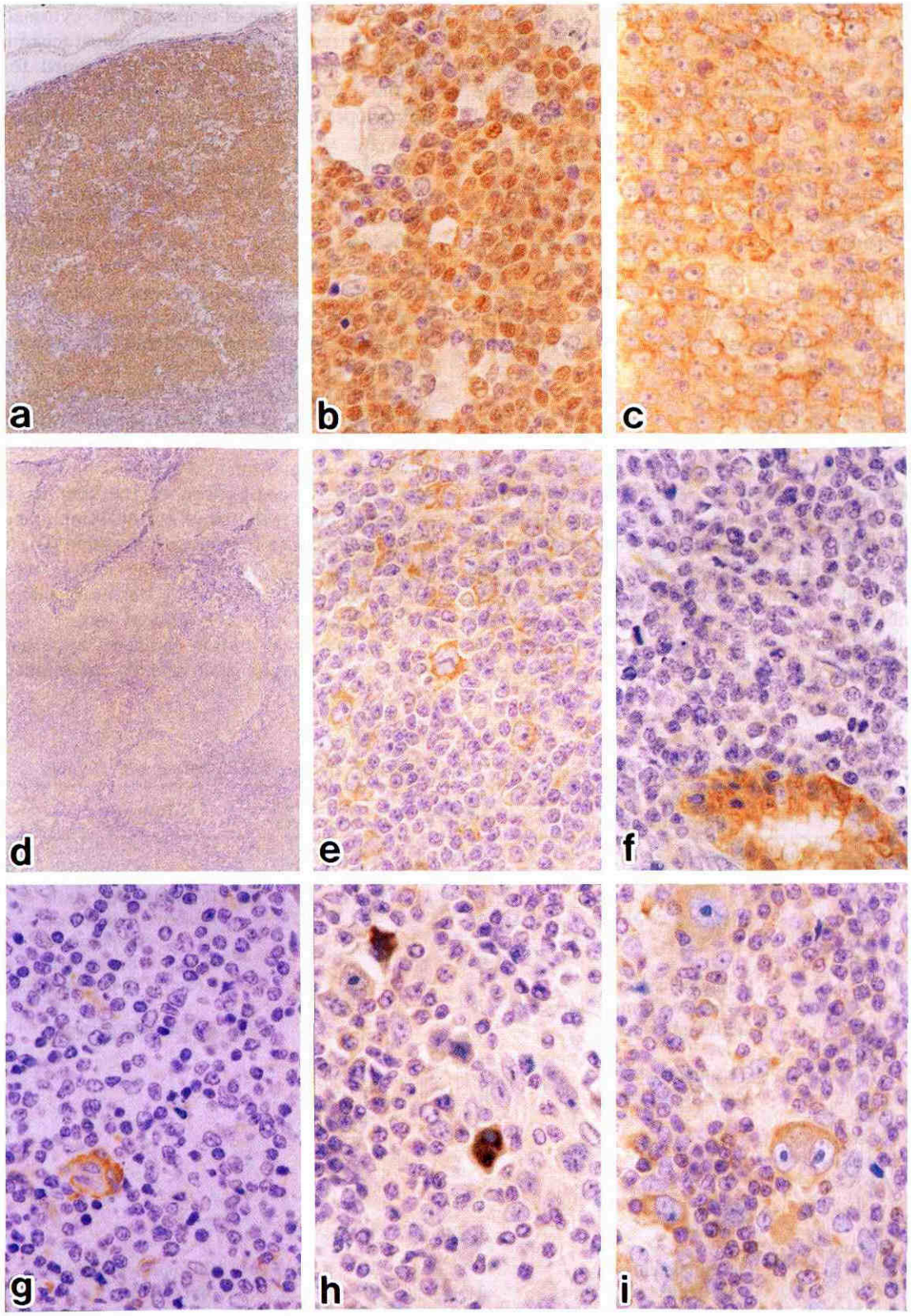
One hundred and six specimens of 104 cases of hematopoietic malignancies or reactive lymphoid processes were obtained as routine diagnostic materials at the histopathology laboratory in the Aichi Cancer Center Hospital, Nagoya. The histologic diagnosis was based on conventional hematoxylin-stained, formalin-fixed, paraffin-embedded sections. Subclassification of the malignant lymphomas was according to the updated Kiel classification[6] and the working formulation.[22] Immunophenotyping was performed either on cell suspensions prepared at the time of biopsy by flow cytometric analysis or on frozen sections by immunohistochemistry.[23] Immunoperoxidase staining was performed for detection of the PRAD1/cyclin D1 protein, using the indirect immunoperoxidase technique.[24] The primary antibody used for staining of sections was IgG2a monoclonal antibody, 5D4, prepared against recombinant human cyclin D1. The generation of 5D4 and its specificity are described elsewhere.[21] For formalin-fixed, paraffin-embedded sections, we applied the microwave oven heating technique,[25] which has been shown to be effective for the retrieval of masked epitopes of antigens. Peroxidase activity was detected by incubation with a solution of diaminobenzidene and counterstained with hematoxylin. Preliminary study showed that not only frozen but also formalin-fixed paraffin-embedded sections were positively stained with the 5D4 antibody, so the latter was used for the immunohistologic staining study. Semi-quantitative estimation of nuclear and cytoplasmic staining intensity was based on comparison of the staining intensity of malignant cells with that of normal lymphoid organs, and was classified into four grades; negative (0), weak (1+), intermediate (2+), and strong (3+). Weak staining (1+) was barely detectable, but more intense than the mantle zone in normal lymphoid tissue.

A nodular distribution indicates large aggregates of weakly to strongly positive cells surrounded by a rim

Table I. Expression of PRAD1/Cyclin D1 Protein in Reactive Lymph Nodes, Spleen and Thymus

Case No.	PRAD1 distribution	Intensity	Staining pattern	Histology
Reactive lymph nodes				
1-A	Germinal centers	0−1+	C	Reactive hyperplasia
1-B		0		Reactive hyperplasia
1-C	Germinal centers	1+	C	Reactive hyperplasia
1-D		0		Reactive hyperplasia
1-E		0		Reactive hyperplasia
1-F	Germinal centers	1+	C	Reactive hyperplasia
	scattered centroblasts	2+	C	
1-G	Germinal centers	1+	C	Reactive hyperplasia
Spleen				
1-H	Germinal centers	1+	C	
	Marginal zones	1−2+	C	
1-I		0		
1-J		0		
Thymus				
1-K		0		
1-L	Cortex	1+	C	
	Medulla	1+	C	
Thyroid				
1-M	Germinal centers	1+	C	Chronic lymphocytic
	scattered centroblasts	2+	C	thyroiditis

Abbreviation: C, cytoplasmic staining.


a
b
c
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i

of negative or more weakly positive cells. Follicular lymphomas and some mantle cell lymphomas demonstrate this pattern of distribution. A uniform pattern describes the situation that almost all lymphoid cells express PRAD1/cyclin D1 at a similar intensity, either weak or strong. "Uniform and nodular" refers to the presence of both patterns in different areas. Scattered distribution refers to the presence of PRAD1/cyclin D1 in only an occasional cell within a lymphoid proliferation. "Nuclear" staining pattern indicates nuclear expression with and without cytoplasmic positivity, while "cytoplasmic" staining implies cytoplasmic localization without nuclear staining.

Semi-quantitative evaluation was performed independently by two of us (SN and TK). In the occasional instance of a discrepancy, the slides were reviewed by a third observer to reach a consensus opinion.

RESULTS

Normal or reactive lymphoid follicles and inflammatory infiltrates of the thyroid gland were analyzed to provide a basis for comparison with the malignant process. We assessed the intensity and distribution of PRAD1/cyclin D1 expression in seven reactive lymph nodes (Table I). PRAD1/cyclin D1 was weakly positive (0−1+ or 1+) in the follicular centers of four cases, and was barely present in mantle zones and interfollicular or T-cell zones. A similar staining pattern was seen in one of three spleens, in that germinal centers were stained, but mantle zones were negative. Marginal zones were also weakly to intermediately positive in one case. In one of two thymuses, PRAD1/cyclin D1 protein was weakly detectable. Non-malignant inflammatory infiltrates in the thyroid gland expressed a weak to intermediate level (1+ to 2+) of PRAD1/cyclin D1 protein in germinal centers. Their staining appeared to be intracytoplasmic, and not nuclear.

PRAD1/cyclin D1 was strongly expressed (2+ to 3+) in 17 of 20 mantle cell lymphomas (Table II), and 17 of the positive cases were characteristic in their staining pattern, which appeared to be either predominantly nuclear or both nuclear and cytoplasmic (Fig. 1a, b). Three of these positive cases had the chromosome abnormality of t(11;14)(q13;q32) or t(11;22)(q13;q11), but chromosomal analysis was not conducted in the rest of the cases. The staining often demonstrated a nodular pattern, and the areas of PRAD1/cyclin D1 expression corresponded to those of tumor cell involvement. The positive neoplastic nodules (2+ to 2−3+) of three cases sometimes demonstrated PRAD1/cyclin D1-weak residual follicular centers that were compressed by the expanding population of lymphoma cells. This pattern of PRAD1 staining was the inverse of that demonstrated by normal mantle zones, in which PRAD1 was undetected. In three mantle cell lymphomas, PRAD1/cyclin D1 expression was weak (1+) to intermediate (2+) and was cytoplasmic.

Among 15 diffuse B-cell lymphomas (12 centroblastic, two immunoblastic, one large cell anaplastic and one lymphoblastic type) arising in lymph nodes, intense (2+ to 3+) PRAD1/cyclin D1 expression was demonstrated by eight with the cytoplasmic staining pattern (Fig. 1c), but no nuclear staining was seen (Table III). In six of the remaining cases, PRAD1/cyclin D1 was weak to intermediate (1+ or 1–2+) in intensity, and in only one case was it absent (0).

Six (60%) of 10 nodal follicular lymphomas (four CD5−, 10−, three CD5−, 10+, and three unexamined) showed PRAD1/cyclin D1-weakly positive (1+) malignant follicles (Fig. 1d) with cytoplasmic staining, which were surrounded by a rim of negative cells, while the other four showed no or very weak reactivity with the 5D4 antibody (Table IV). In several neoplastic follicles of two cases, a small number of single positive centroblasts (2+) were scattered, but their positivity appeared to be intracytoplasmic (Fig. 1e).

In contrast to the nodal lymphomas, many extranodal lymphomas of B-cell origin expressed much lower levels of PRAD1 protein (Table V). Immunohistochemical

Fig. 1. Immunohistochemical staining of PRAD1/cyclin D1 protein in malignant lymphomas. (a) Mantle cell lymphoma. Note the nodular pattern of positively stained lymphoma cells. (magnification ×20) (b) Higher magnification of Fig. 1a. The intense (3+) positive staining appears to be predominantly nuclear, but cytoplasmic staining is also observed in a proportion of the tumor cells. PRAD1/cyclin D1 is absent in the macrophages admixed with the tumor cells. (magnification ×400) (c) Nodal diffuse large B-cell lymphoma. The staining is intense (2–3+), and the expression sppears to lie in the cytoplasm of the lymphoma cells, but not macrophages. (magnification ×200) (d) Nodal follicular lymphoma. PRAD1/cyclin D1 protein was uniformly weak (1+). (magnification ×40) (e) Higher magnification of Fig. 1e. The antibody shows intermediate (2+) cytoplasmic staining of scattered centroblasts in the neoplastic follicles. (magnification ×400) (f) Diffuse large B-cell lymphoma of the stomach. The antibody reacts with the glandular epithelium, but not with lymphoma cells (0). (magnification ×400) (g) Monocytoid B-cell lymphoma of the tonsil. The antibody shows negative staining (0) in the lymphoma cells. (magnification ×400) (h, i) Hodgkin's disease, mixed type. Only a few Reed-Sternberg cells (mummified cells ?) (h) show nuclear staining pattern, while some (i) demonstrated intermediate (2+) cytoplasmic positivity. (magnification ×400) (immunoperoxidase, counterstained with hematoxylin).

Table II. Expression of PRAD1/Cyclin D1 Protein in Mantle Cell Lymphoma

Case No.	PRAD1 distribution	Intensity	Staining pattern	Phenotype (karyotype)
2-A	Uniform	2+	N	CD5+,10−,IgM.λ
2-B	Uniform	2+	N/C	CD5+,10−,IgM.κ
2-C	Uniform	1+	C	CD5+,10−,IgM.λ
2-D	Uniform	3+	N	CD5+,10−,IgG.M.κ
2-E	Uniform	1−2+	N	CD5+,10−,IgG.M.λ
2-F	Uniform	3+	N	CD5+,10−,IgM.λ t(11;14)(q13;q32)
2-G	Uniform	2+	N	CD5+,10−,IgG.M.D.λ
2-H	Uniform	3+	N	CD5+,10−,IgG.M.A.λ
2-I	Uniform	2+	N/C	CD5+,10−,IgG.λ
2-J	Uniform	3+	N	CD5+,10−,IgM.D.κ
2-K	Uniform	2+	N/C	CD5+,10−,IgG.M.A.D.λ
2-L	Uniform	2−3+	N	CD5+,10−,IgM.λ
2-M	Nodular and uniform	2+	N	CD5+,10−,IgM.λ
2-N	Nodular and uniform	2+	N/C	CD5+,10−,IgM.D.κ
	Nodular and uniform[a)]	2−3+	N/C	t(11;14)(q13;q32)
2-O	Uniform	2−3+	N/C	CD5+,10−,IgG.M.λ
2-P	Uniform	2+	N/C	CD5+,10− t(11;22)(q13;q11)[b)]
2-Q	Uniform	2+	C	CD5+,10−,IgM.D.λ
2-R	Nodular and uniform	2+	N/C	CD5+,10−,IgM.D.λ
2-S	Nodular and uniform	2+	N/C	CD5+,10−,IgM.D.λ
2-T	Nodular and uniform	1+	C	CD5+,10−,IgM.λ

Abbreviation: C, cytoplasmic staining; N, predominantly nuclear staining; N/C, both nuclear and cytoplasmic staining.
a) Second biopsy of the relapsed lymph node.
b) See references 18 and 19.

Table III. Expression of PRAD1/Cyclin D1 Protein in Nodal Diffuse Large Cell Lymphoma of B-Cell Phenotype

Case No.	PRAD1 distribution	Intensity	Staining pattern	Histology	Phenotype
3-A	Uniform	1−2+	C	Large cell, diffuse; centroblastic	CD5−,10−,IgM.λ
3-B	Uniform	2+	C	Large cell, diffuse; centroblastic	CD5−,10−,IgG.κ
3-C		0		Large cell, diffuse; centroblastic	CD5−,10−,IgM.D.λ
3-D	Uniform	1−2+	C	Large cell, diffuse; centroblastic	CD5−,10−,IgM.D.κ
3-E	Uniform	1−2+	C	Large cell, diffuse; centroblastic	CD5−,10−
3-F	Uniform	3+	C	Large cell, diffuse; centroblastic	CD5−,10−,IgM.λ
3-G	Uniform	2+	C	Large cell, diffuse; centroblastic	
3-H	Uniform	2−3+	C	Large cell, diffuse; centroblastic	CD5−,10+/−,λ
3-I	Uniform	2−3+	C	Large cell, diffuse; centroblastic	
3-J	Uniform	1−2+	C	Large cell, diffuse; centroblastic	
3-K	Uniform	1−2+	C	Large cell, diffuse; centroblastic	
3-L	Uniform	2−3+	C	Immunoblastic	CD5−,10+/−,IgM.κ
	Uniform[a)]	2+	C		
3-M	Uniform	1−2+	C	Immunoblastic	
3-N	Uniform	3+	C	Immunoblastic; large cell anaplastic	CD5−,10−
3-O	Uniform	2−3+	C	Lymphoblastic	CD10+,19+

Abbreviation: C, cytoplasmic staining.
a) Second biopsy of the relapsed lymph node.

staining of extranodal lymphomas with 5D4 antibody exhibited no reactivity in 10 (59%) of 17 cases (0) (Fig. 1f). Similarly, four cases of monocytoid B-cell lymphoma,[2, 6, 26)] which was regarded as the nodal equivalent of malignant lymphoma of mucosa-associated lymphoid tissue,[2, 6, 27, 28)] showed absent or weak reactivity (Fig. 1g).

Table IV. Expression of PRAD1/Cyclin D1 Protein in Nodal Follicular Lymphoma

Case No.	PRAD1 distribution	Intensity	Staining pattern	Histology	Phenotype
4-A	Nodular	1+	C	Mixed, follicular; centroblastic-centrocytic	CD5−,10−,IgM.κ
4-B	Nodular	0−1+	C	Mixed, follicular; centroblastic-centrocytic	CD5−,10−,IgD.κ
4-C	Nodular	1+	C	Mixed, follicular; centroblastic-centrocytic	CD5−,10−,IgA.G.λ
4-D	Nodular	1+	C	Mixed, follicular; centroblastic-centrocytic	CD5−,10+,IgM.λ
4-E	Nodular and scattered centroblasts	1+ 2+	C	Mixed, follicular; centroblastic-centrocytic	CD5−,10+,IgA.G.M.λ
4-F	Nodular	0−1+	C	Mixed, follicular; centroblastic-centrocytic	
4-G	Nodular	1+	C	Mixed, follicular; centroblastic-centrocytic	CD5−,10−,IgG.M.D.κ
4-H	Nodular	1+	C	Mixed, follicular; centroblastic-centrocytic	CD5−,10+,IgG.M.κ
4-I	Only a few scattered centroblasts	2+	C	Small cleaved, follicular; centroblastic-centrocytic	
4-J	Nodular	0−1+	C	Small cleaved, follicular; centroblastic-centrocytic	

Abbreviation: C, cytoplasmic staining.

Table V. Expression of PRAD1/Cyclin D1 Protein in Extranodal Lymphoma of B-Cell Phenotype and Monocytoid B-Cell Lymphoma of the Tonsil and Lymph Nodes

Case No.	PRAD1 distribution	Intensity[a]	Staining pattern	Histology	Site
5-A		0		Small cleaved, diffuse; low-grade MALT	Stomach
5-B		0		Small cleaved, diffuse; low-grade MALT	Stomach
5-C	Uniform	1+	C	Small cleaved, diffuse; low-grade MALT	Stomach
5-D	Scattered cells with plasmacytoid differentiation	2+	C	Small cleaved, diffuse; low-grade MALT	Stomach
5-E	Scattered cells with plasmacytoid differentiation	2+	C	Mixed, diffuse; high-grade MALT	Stomach
5-F		0		Large cell, diffuse; high-grade MALT	Stomach
5-G		0		Large cell, diffuse; high-grade MALT	Stomach
5-H	Uniform	1−2+	C	Large cell, diffuse; high-grade MALT	Stomach
5-I		0		Large cell, diffuse; high-grade MALT	Stomach
5-J		0		Large cell, diffuse; centroblastic	Stomach
5-K		0		Large cell, diffuse; centroblastic	Stomach
5-L		0		Large cell, diffuse; centroblastic	Stomach
5-M		0		Small non-cleaved; Burkitt's	Stomach
5-N	Nodular	0−1+	C	Small cleaved, follicular; centroblastic-centrocytic	Jejunum
5-O		0−1+	C	Small cleaved, follicular; centroblastic-centrocytic	Jejunum
5-P		0		Small cleaved, diffuse; low-grade MALT	Skin
5-Q	Uniform	2+	C	Large cell, diffuse; centroblastic	Conjunctiva
Monocytoid B-cell lymphoma					
5-R		0		Mixed; monocytoid	Tonsil
5-S	Uniform	1+	C	Mixed; monocytoid	Lymph node
5-T		0		Mixed; monocytoid	Lymph node
5-U		0		Mixed; monocytoid	Tonsil

a) Refers to the staining of lymphoma cells.
Abbreviations: low-grade MALT, low-grade malignant B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) type; high-grade MALT, high-grade malignant B-cell lymphoma of MALT type with a low-grade malignant component; monocytoid, monocytoid B-cell lymphoma.

Table VI. Expression of PRAD1/Cyclin D1 Protein in T-Cell Lymphomas

Case No.	PRAD1 distribution	Intensity	Staining pattern	Histology
6-A		0		Lymphoblastic
6-B	Uniform	1+	C	Lymphoblastic
6-C		0		Lymphoblastic
6-D		0		Lymphoblastic
6-E		0		Mixed; pleomorphic medium and large cell
6-F	Scattered	0−1+	C	Large cell; pleomorphic large cell
6-G	Uniform	1−2+	C	Large cell; pleomorphic large cell
6-H	Uniform	2+	C	Large cell; large cell immunoblastic
6-I	Variable	1+	C	Mixed; lymphoepithelioid (Lennert's)
6-J	Uniform	2+	C	Mixed; angioimmunoblastic
6-K		0		Mixed; T-zone
6-L		0		Immunoblastic; large cell anaplastic
6-M	Uniform	1+	C	Immunoblastic (non-T non-B); large cell anaplastic
6-N	Uniform	0−1+	C	Immunoblastic; large cell anaplastic
6-O		0		Immunoblastic; large cell anaplastic

Abbreviation: C, cytoplasmic staining.

Immunohistochemical staining of four T-lymphoblastic lymphomas with 5D4 antibody exhibited 0 or 1+ reactivity (Table VI). Peripheral T-cell lymphomas exhibited negative to intermediate staining (0 to 2+). The intensity of PRAD1/cyclin D1 throughout the peripheral T-cell lymphomas was variable and usually not so intense as in most nodal diffuse B-cell lymphomas.

Immunohistochemistry on lymph nodes from six cases of Hodgkin's disease showed Reed-Sternberg and mononuclear Hodgkin's cells expressing variable degrees of PRAD1/cyclin D1 protein from absent to intermediate (0 to 2+) (Table VII). Only one case contained a small population of cells expressing nuclear staining of PRAD1/cyclin D1 besides the cells with cytoplasmic pattern and with no staining (Fig. 1h, i).

Langerhans cell histiocytosis manifested in skin and sinus histiocytosis with massive lymphadenopathy expressed intermediate levels (1−2+ to 2+) of PRAD1 (Table VIII). Granulocytic sarcoma showed weak (1+) staining.

The immunohistochemical findings in malignant lymphomas are summarized in Table IX.

Table VII. Expression of PRAD1/Cyclin D1 Protein in Hodgkin's Disease

Case No.	Intensity[a)]	Staining pattern	Histology
7-A	1+	C	Lymphocyte predominance
7-B	0		Nodular sclerosis
7-C	1−2+	C	Mixed
7-D	1+	C	Mixed
7-E	2+	C	Mixed
7-F	2+	C	Mixed
7-G	2+	N/C	Mixed

a) Refers to the staining of Reed-Sternberg and Hodgkin's cells.
Abbreviations: C, cytoplasmic staining; N/C, both nuclear and cytoplasmic staining.

DISCUSSION

Recently, the regulated synthesis and destruction of cyclin proteins have been suggested to be critical for proper cell-cycle control in eukaryotes, and cyclin D1 protein appears to be required for cell-cycle progression from the G1 to S phase.[29–31)] It is interesting that cyclin D1 has been found to be overexpressed in a variety of human tumors including breast and esophageal cancers,[32)] and that transfection of an activated copy of its gene contributes to oncogenic transformation *in vitro*, particularly in cooperation with ras mutations.[30)] Moreover, cyclin D1 transgene was recently reported to collaborate in lymphomatogenesis with the myc gene.[33)] These data suggest that the overexpression of cyclin D1 protein deregulates the cell-cycle control and leads to transformation, in cooperation with other oncogenes. This implies that cyclin D1 functions as an oncoprotein and links disturbances in the cell-cycle machinery to the process of cell transformation.[30, 31, 33)]

The overexpression of PRAD1/cyclin D1 in mantle cell lymphomas has been demonstrated by several authors using molecular probes.[16–20)] It has only recently become possible to investigate the immunohistochemical distribution of the PRAD1/cyclin D1 protein using a monoclonal antibody.[21, 32)] All methods based on extract-

Table VIII. Expression of PRAD1/Cyclin D1 Protein in Other Hematologic Malignancies

Case No.	PRAD1 distribution	Intensity	Staining pattern	Histology
8-A	Uniform	2+	C	Langerhans cell histiocytosis
8-B	Uniform	1−2+	C	Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman)
8-C	Scattered	1+	C	Granulocytic sarcoma (AML)

Abbreviation: C, cytoplasmic staining.

Table IX. Summary of Expression of PRAD1/Cyclin D1 Protein in Lymphoproliferative Disorders

Type of lymphoma	Staining pattern[a]		Intensity[a]	
	Nuclear	Cytoplasmic	2+ to 3+	0 to 1−2+
Non-Hodgkin's lymphoma				
Mantle cell lymphoma	17/20 (85)	3/20 (15)	18/20 (90)	2/20 (10)
Follicular	0/10 (0)	10/10 (100)	2/10 (20)	8/10 (80)
Diffuse				
B lineage, nodal[b]	0/15 (0)	14/15 (93)	8/15 (53)	7/15 (47)
B lineage, extranodal	0/17 (0)	7/17 (41)	3/17 (18)	14/17 (82)
B lineage, monocytoid	0/4 (0)	1/4 (25)	0/4 (0)	4/4 (100)
T lineage	0/15 (0)	8/15 (53)	2/15 (13)	13/15 (87)
Hodgkin's disease	1/7 (14)	5/7 (71)	3/7 (43)	4/7 (57)

a) Cases positive/cases studied (%).
b) Excluding monocytoid B-cell lymphoma.

ing total nucleic acids or protein from tumor tissue specimens can only provide a tissue-averaged figure. In contrast, immunohistochemical staining reveals the localization and levels of protein within individual tumor cells, and is not subject to underestimation due to the presence of normal cells in the tissue. In the previous study,[21] we preliminarily showed that the PRAD1/cyclin D1 protein is detectable as nuclear staining in neoplastic cells of mantle cell lymphoma, but not in lymphomas of the other types, based on a small number of cases. The present immunolocalization of PRAD1/cyclin D1 protein confirmed predominantly nuclear or both nuclear and cytoplasmic staining patterns in 17 (85%) of 20 mantle cell lymphomas; this topography is opposite to that of the normal reactive mantle zones. Nuclear staining was not observed in any of the other 61 non-Hodgkin's lymphomas, including those which pose a differential diagnostic problem from mantle cell lymphoma in a paraffin section, e.g. lymphoblastic lymphoma, centrocytic-centroblastic lymphoma, and pleomorphic T-cell lymphoma. It is noteworthy that the present monoclonal antibody afforded strong nuclear immunostaining in three cases of mantle cell lymphoma, where the 11q13 translocation has been proven.[18, 19, 21] These findings suggest that the nuclear staining pattern might possibly provide a means to detect lymphomas bearing 11q13 translocation or amplification. In the present study, 85% of mantle cell lymphomas exhibited a nuclear staining pattern. This percentage is in reasonable agreement with a recent report that 75–100% of mantle cell lymphomas contain significantly elevated levels of cyclin D1 RNA.[16, 20] Three cases of mantle cell lymphomas did not demonstrate nuclear staining, indicating that a small subset may arise without cyclin D1 overexpression. It should also be noted that 3 to 4% of chronic lymphocytic leukemia and multiple myeloma possess 11q13 translocation,[10] and indeed, we have shown that a myeloma cell line with this abnormality exhibits nuclear staining.[21] However, it is still uncertain whether these rare cases have any relationship to mantle cell lymphoma. Careful molecular analysis of the *PRAD1*/cyclin D1 gene in the present series of lymphomas is in progress in an attempt to establish the basis of the observed cyclin D1 expression.

Another finding of interest was the demonstration of the cytoplasmic pattern of immunoreactivity without nuclear staining in 40 of 61 non-Hodgkin's lymphomas excluding mantle cell lymphoma. The staining intensity was variable and seemed to depend on the subcategory of lymphoma. A high incidence of positive cases was found in nodal follicular and diffuse B-cell lymphomas as compared with T-cell and extranodal B-cell lymphomas

(Table IX). There was similarity in staining quality between the cells of the nodal diffuse large-B-cell lymphomas and the centroblasts in follicular lymphomas. Monocytoid B-cell lymphomas demonstrated a similar incidence and intensity to extranodal B-cell lymphomas. The similarity in weak or absent PRAD1/cyclin D1 staining of extranodal and monocytoid B-cell lymphomas might be correlated with the proximity in cell lineage and the similarity in the indolent clinical course of both diseases.[6] It should be pointed out that staining intensity comparable to that observed in tumors was never seen in reactive conditions. The cytoplasmic pattern of staining was recently reported to be rare in breast cancers using another monoclonal antibody (DCS-6) against recombinant human cyclin D1, although the cytoplasm was stained in some of the cases with nuclear staining.[32] Baldin *et al.*[29] also found that cyclin D1 is a nuclear protein which accumulates in the nucleus in G1 phase and is immunohistochemically recognized as a low-intensity specific cytoplasmic staining in quiescent cells. The different antibodies used in these studies may have influenced the results, and further investigation of the significance of cytoplasmic staining of PRAD1/cyclin D1 is still needed.

Only one case of Hodgkin's disease revealed a heterogeneous population of Reed-Sternberg cells expressing nuclear, intracytoplasmic and absent staining patterns of PRAD1/cyclin D1; the significance of this phenomenon still remains unclear.

It is concluded that immunohistologic staining for PRAD1/cyclin D1 protein may be helpful in distinguishing mantle cell lymphoma from the other types of non-Hodgkin's lymphoma. The present study also documents the incidence and intensity of cytoplasmic positivity among nodal and extranodal B-cell, and T-cell lymphomas.

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