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Pax5 expression in Non-Hodgkin's Lymphomas and Acute Leukemias

The *Pax5* gene encodes the B-cell-specific activator protein which is a key regulator in development and differentiation of B-cell. We studied the expression of Pax5 in hematologic malignancies to evaluate the diagnostic utility as a B cell marker. Materials included 70 B cell lymphomas, 26 T cell lymphomas, 53 acute leukemias, and 6 multiple myelomas (MMs). Representative areas from the paraffin-embedded tissues were selected for tissue microarray, and the expressions of Pax5 was immunohistochemically evaluated. Pax5 was strongly expressed in most of the B cell lymphomas; 44 of 47 diffuse large B cell lymphomas (93.6%), 15 of 16 marginal zone B cell lymphomas (93.8%), all 3 mantle cell lymphomas, 2 follicular lymphomas, and 2 Burkitt's lymphomas (100%). However, Pax5 was expressed in only one of 26 T cell lymphomas. Among leukemias, it was expressed in 10 of the 14 B acute lymphocytic leukemias (ALLs) (72.4%), but also in 3 of the 6 T ALLs (50%), 13 of the 26 acute myelogenous leukemias (AMLs) (50%) and in all 3 ALL arising in chronic myelogenous leukemias and 4 mixed B ALL and AML. In MMs, Pax5 was negative in all cases. We concluded that Pax5 is very useful B cell marker in classification of lymphomas, but not of acute leukemias.

Key Words : *B cell Activation Antigen; Lymphoma, Non-Hodgkin; Leukemia, B-cell, Acute; Leukemia, Myelocytic, Acute; Multiple Myeloma*

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INTRODUCTION

A variety of chromosomal alterations, such as translocation, deletion or mutation, play an important role in the pathogenesis of many human hematologic malignancies (1-3), and the detection of proteins encoded by altered chromosomes has been used in diagnosis of hematologic disorders (4).

Pax5 gene is a member of the paired-box *Pax* gene family, and encodes the transcription factor, Pax5, and is also known as B-cell-specific activator protein (BSAP) which is detected in the developing central nervous system, in the adult testis, and in the cells of the B-lymphoid lineage (5). In B cell development, *Pax5* gene transcription is initiated in pro-B (pre-BI) cell stage, and expressed through the pre-B- and mature B cells, but not in plasma cells (5, 6). It activates expression of *mb*-1, LEF-1, N-*myc*, and also the *CD19* gene (5, 7, 8). About half of the lymphoplasmacytic lymphomas are associated with t(9;14) (p13;q32), which leads to juxtaposition of *Pax5* gene to IgH gene (3, 9, 10). Recently, polyclonal and monoclonal antibodies for Pax5 protein were used in routinely processed lymphoid neoplasia and reported to be an excellent pan-B and pan-pre-B cell marker, which are not associated with chromosomal alterations (11, 12).

In this study, the expression of Pax5 was immunohistochemically detected in non-Hodgkin's lymphomas and in leukemias to evaluate its usefulness as B cell marker.

MATERIALS AND METHODS

Materials

Non-Hodgkin's lymphomas and acute leukemias were selected from the Pathology File in the Department of Pathology, Anam Hospital of Korea University Medical College, Seoul, Korea. The tissues were routinely processed with 10% buffered formalin fixation and paraffin embedding. The diagnosis and classification of lymphomas were dependent upon the routine H-E stained slides and immunohistochemistry. The diagnosis was supplemented by IgH and TCR rearrangement studies and followed the WHO classification. In leukemias, the immunophenotyping was performed by flow cytometry, using a panel of monoclonal antibodies, and bone marrow core biopsies were used for this study. All slides were reviewed, and appropriate areas were marked and selected for the tissue microarray. Agar blocks for tissue microarray were prepared as follows. Agar (4 g) was dissolved into 100 mL distilled water and boiled in microwave for 2 min; it was solidified in the cast, and was routinely processed as tissue sample; the agar was embedded in paraffin; the selected area of the each case was punch-holed using skin punch biopsy tool (2 mm in diameter) and was transplanted into the agar block, which was also punch-holed with the same biopsy tool; and after all cases were re-embedded into the agar block, the block

was incubated at 37°C for 30 min to mould the tissues into paraffin.

The selected cases included 53 acute leukemias (14 B acute lymphocytic leukemias [ALLs], 6 T ALLs, 26 acute myelogenous leukemias [AMLs], 3 ALLs arising in chronic myelogenous leukemia [CML], and 4 mixed B ALL and AML), 70 B cell lymphomas (47 diffuse large B cell lymphomas [DLBCL], 16 marginal zone B cell lymphomas [MZBCL], 2 follicular lymphomas [FL], 2 Burkitt's lymphomas [BL], and 3 mantle cell lymphomas [MCL]), 26 T cell lymphomas (9 not otherwise specified [NOS], 6 NK/T cell lymphomas [NK/T], 3 angioimmunoblastic lymphomas [AIL], 3 anaplastic lymphomas [AL], and 5 lymphoblastic [LB]). Six cases of multiple myeloma [MM] were also included.

Immunohistochemical Staining

For immunohistochemical staining, 4-μm thick section from microarray blocks were deparaffinized and rehydrated. After endogenous peroxidase activity was eliminated by incubation with 3% H_2O_2 in methanol, the retrieval of antigen was done by placing the slides for 2 min in a pressure cooker (103 Kpa) containing 0.01 M sodium citrate buffer (pH 6.0). The slides were then incubated with the primary monoclonal antibodies, Pax5 (1:50, Transduction Laboratories, Lexington, KY, U.S.A.) for one hour at room temperature. After incubating for 30 min with biotinylated link antibody, the sections were re-incubated with streptavidin-peroxidase complex for 30 min. Immunostaining was visualized by using 3, 3′ diaminobenzidine. The sections were counterstained with hematoxylin. As a negative control, 0.1 M Tris buffer (pH 7.6) replaced the primary antibody, and the tonsil and lymph node were used as positive control.

Immunohistochemical result for Pax5 protein was interpreted as positive, when the nucleus of neoplastic cells was positive.

RESULTS

Pax5 expression was in the nuclei of B lymphocytes of normal tonsil and lymph node. The germinal center cells were found positive, but mantle cells were the strongest. T zone lymphocytes, plasma cells, endothelial cells, and macrophages were found negative. In B cell lymphomas, all BLs (2/2), MCLs (3/3) and FLs (2/2), 44 of 47 DLBCLs (93.6%) and 15 of 16 MZBCLs (93.8%) were found positive. The nuclear staining in B cell lymphomas was diffusely strong in most of the neoplastic cells, whereas the staining was patch, mainly in the nuclei of the small lymphocytes and centrocyte-like cells, but not in plasma cells in MZBCLs. Six MMs were found entirely negative. Pax5 was found negative in all T cell lymphomas except in one of the 5 LBs (20%). In LBs, the positive cells were found in less than 50% of the neoplastic cells. Pax5 was expressed in 10 of the 14 B ALLs (72.4%), 3 of 6 T ALLs (50%), 13 of 26 AMLs (50%), and in all 3 ALLs arising in CML and 4 mixed B ALL and AML (100%).

DISCUSSION

Pax5 protein is known to be a B-cell specific marker, which is expressed exclusively in normal and neoplastic B cells. The levels of Pax5 expression varied among different B cell subsets and among B cell NHL subtypes. In normal lymphoid tissues, Pax5 expression was found in B lymphocytes, but not in plasma cells (5, 6, 13). In our study, Pax5 expression was mainly seen in the nuclei of B cell follicles, and mantle cells of normal tonsil and lymph node were strong, but T zone lymphocytes, plasma cells, endothelial cells, and macrophages were found negative. Among B cell lymphomas, strong expression of Pax5 was seen in most of MCL, DLBCL, FL, BL, and MZBCL. Interestingly, the staining in MZBCL was patch and was mainly limited in the nuclei of the small lymphocytes and centrocyte-like cells, but not in plasma cells. The study done by Foss et al. (14) showed that all B cell lymphomas were found positive for Pax5, whereas all T cell lymphomas were found negative, though two of the 5 plasmacytomas showed a focal positive reaction by in situ hybridization. The results of the studies of Krenacs et al. (11) and Torlakovic et al. (12), which included a relatively large numbers of a variety of histologic subtypes, showed similar results. However, in our study, Pax5 was expressed in one T cell LB lymphoma, 3 of 6 T ALLs, and 13 of 26 AMLs. The expression of Pax5 was not related with CD19 expression, which was studied in flow cytometry. Our

Table 1. Expression of Pax5 in Non-Hodgkin's Lymphomas and Leukemias

Diagnosis		Total Cases	No. of Positive Cases (%)
B cell neoplasm	DLBCL	47	44 (93.6)
	MZBCL	16	15 (93.8)
	FL	2	2 (100)
	Lymphoblastic	2	2 (100)
	MCL	3	3 (100)
	MM	6	0 (0)
T cell neoplasm	NOS	9	0 (0)
	NK/T	6	0 (0)
	AIL	3	0 (0)
	Anaplastic	3	0 (0)
	Lymphoblastic	5	1 (20)
Leukemia	Early PreB ALL	7	5 (72.4)
	PreB ALL	7	5 (72.4)
	ALL in CML	3	3 (100)
	T ALL	6	3 (50)
	AML	26	13 (50)
	Mixed B ALL and AML	4	4 (100)

DLBCL, Diffuse large B cell lymphoma; MZBCL, Marginal zone B cell lymphoma; FL, Follicular lymphoma; MCL, Mantle cell lymphoma; MM, Multiple myeloma; NOS, Not otherwise specified; AIL, Angioimmunoblastic.

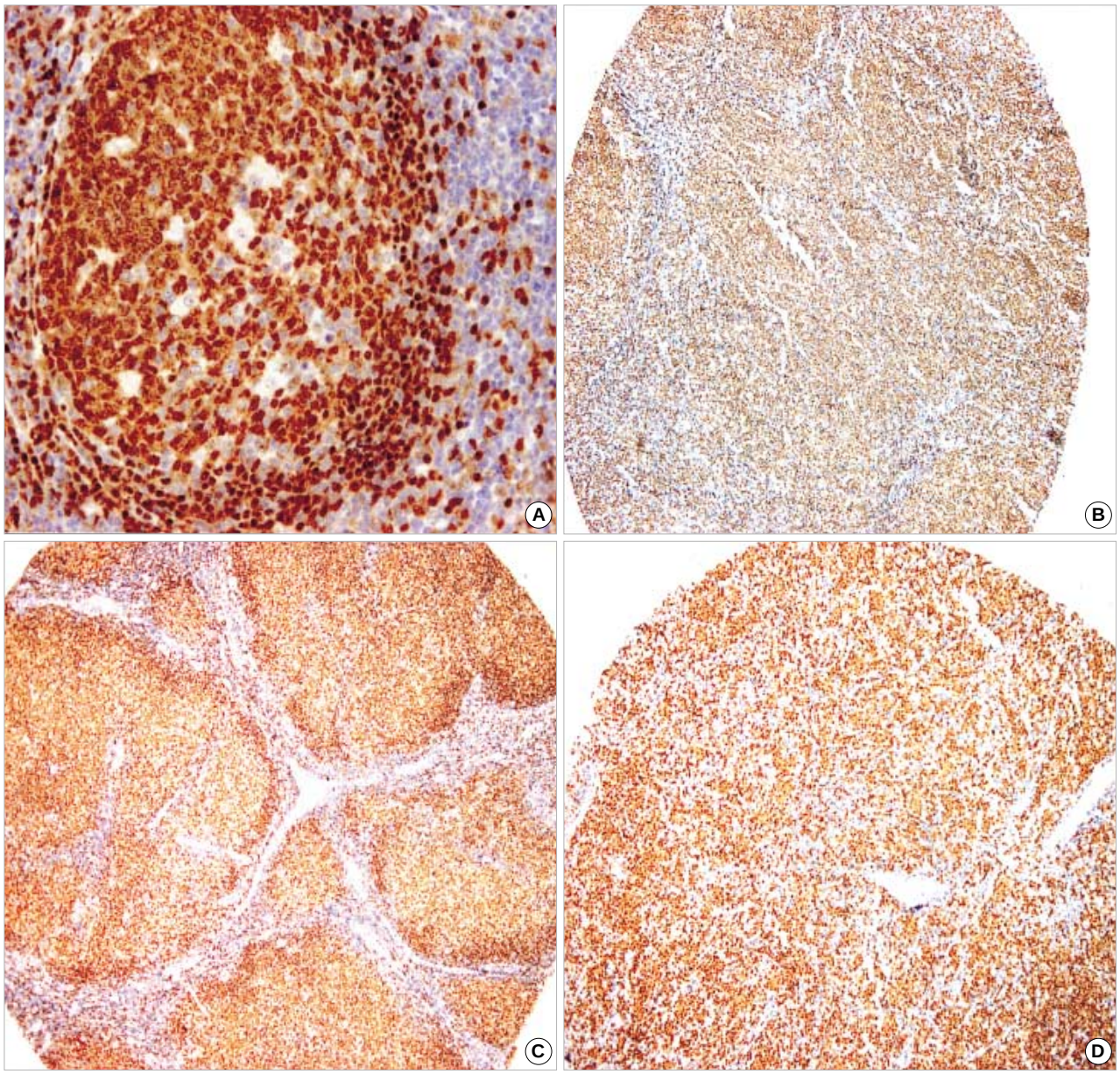

Fig. 1. (A) Germinal center and mantle zone cells are strongly positive for Pax5 in the normal tonsil (×200). (B) In MZBCL, the staining was patch and was mainly in the nuclei of the small lymphocytes (×100). (C) The nodules of FL are positive for Pax5 (×100). (D) DLBCL is diffusely strong positive for Pax5 (×100). *(Fig.1 continued next)*

result on leukemias could not be compared with other results because there were no studies available readily. Pax5 is important for maintaining the identity and the functions of mature B cells in late B-lymphopoiesis, as well as its transcriptional program in early development of B-cell (5-7, 15, 16). The loss of Pax5 leads to reverse B-lineage commitment by converting pro-B cells into hematopoietic progenitors with a broad potential in development (7, 17, 18). Pax5 can be ectopically expressed in a multipotent hematopoietic cell line (18). The expression of Pax5 in hematopoietic progenitor had minimal effects on myeloid differentiation, suggesting that Pax5 is unable to repress myeloid gene transcription in the circumstance of abundant myeloid transcription factors (19). This may explain the expression of Pax5 in AML and T ALL of our studies. Pax5 is also reported to be expressed in Reed-Sternberg and Hodgkin's cells in classic and lymphocyte predominance Hodgkin's lymphomas (11, 12, 20). Recently, Willenbrock et al. (21) presented a case of classic Hodgkin's lymphoma, in which the tumor cells showed clonal rearrangement of T cell receptor β gene, as well as aberrant expression of Pax5 in $CD30^+$ and $CD15^+$ tumor cells. All these findings may explain the expression of Pax5 in the case of T LB lymphoma and of

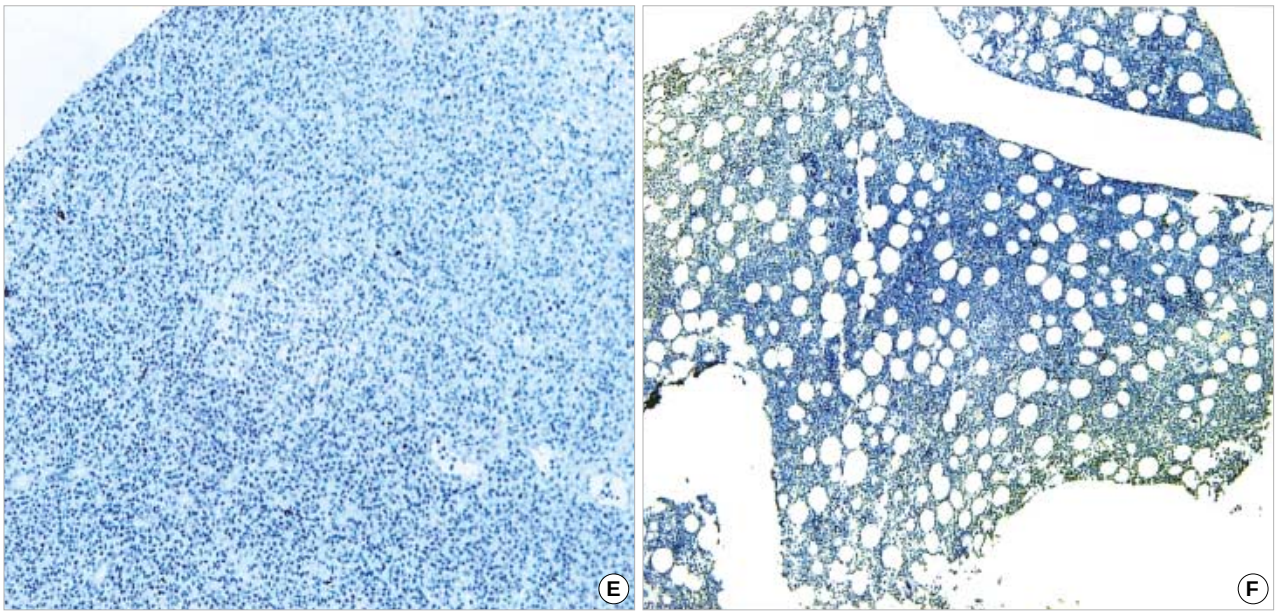

Fig. 1. *(Continued from the previous page)* (E) T cell lymphoma, NOS is found negative for Pax5 (×100). (F) T ALL is found negative for Pax5 (×100).

myeloid leukemias of our study.

In summary, our results showed that Pax5 can be used as a valuable diagnostic marker of B cell lymphomas and of acute lymphoblastic leukemia; however, it has limited value in classification of acute leukemias.

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