Expression Profiling of Transcription Factors in B- or T-Acute Lymphoblastic Leukemia/Lymphoma and Burkitt Lymphoma

Usefulness of PAX5 Immunostaining as Pan–Pre-B-Cell Marker

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Abstract

The optimal use of transcription factors to determine B-lineage specificity in B-acute lymphoblastic leukemia/lymphoma (B-ALL) has not been fully investigated. We undertook an extensive immunohistochemical study of a panel of B-cell transcription factors in B- and T-ALL and Burkitt lymphoma to evaluate those with the best specificity and sensitivity. Tissue microarrays were constructed from 34 B-ALL, 19 T-ALL, and 30 Burkitt lymphoma samples. All 34 (100%) cases of B-ALL expressed PAX5; 32 (94%), BOB.1; 33 (97%), PU.1; 29 (85%), CD79a; 27 (79%), CD22; 2 (6%), CD20; 9 (26%), OCT-2; and 3 (9%), MUM1. Burkitt lymphoma cases were positive for PAX5 (30/30 [100%]), BOB.1 (27/30 [90%]), PU.1 (23/30 [77%]), CD79a (29/30 [97%]), CD22 (14/30 [47%]), CD20 (30/30 [100%]), OCT-2 (23/30 [77%]), and MUM1 (5/30 [17%]). T-ALLs were only positive for PU.1 (15/19 [79%]) and BOB.1 (12/19 [63%]). PAX5 demonstrated better specificity for B-lineage determination than BOB.1 and PU.1 and better sensitivity than CD79a, CD22, and CD20. These findings suggest that PAX5 has the greatest diagnostic usefulness and lineage determination in B-ALL, especially in cases with an inadequate specimen for flow cytometric analysis.

Most hematologic malignancies are subclassified according to cell lineage by incorporating the use of immunophenotyping in lineage assignment. Lineage infidelity of surface or cytoplasmic markers is a well-known phenomenon in lymphomas and leukemias,1–4 which can lead to serious diagnostic difficulties and major impact on patient management. Lineage determination of immature lymphoid malignancies such as B-acute lymphoblastic leukemia/lymphoma (B-ALL) is typically based on flow cytometric studies. In some cases, an adequate specimen is not available for flow cytometric analysis, and, therefore, diagnosis and lineage need to be determined from evaluation of immunohistochemical stains. Lineage determination of B-ALL using immunohistochemical staining on paraffin-embedded tissue has not been studied extensively. Although CD20 is the most commonly used marker for mature B cells, this antibody is neither sensitive nor specific for lineage assignment and often is negative in immature B-cell lymphoid neoplasms.2,3,5 Thus, sometimes a more extensive panel of immunostains is required for accurate diagnosis and appropriate classification.

By using tissue microarray sections, we investigated the expression pattern of a panel of pan–B-cell markers and B-cell transcription factors on 34 B-ALLs, 19 T-ALLs, and 30 Burkitt lymphomas. The aim of the current study was to determine the sensitivity and specificity of these markers in diagnosing immature B-cell neoplasms and to assess the benefit of adding PAX5 and CD22 to the immunohistochemical...
panel used for the separation of immature B-cell–derived neoplasms from their morphologic mimics.

**Materials and Methods**

**Tissue Samples**

After approval by the institutional review board, lymph node paraffin sections or bone marrow clot sections of 34 cases of B-ALL, 19 cases of T-ALL, and 30 cases of Burkitt lymphoma were selected from the files of the Department of Pathology, University of Iowa Hospitals and Clinics, Iowa City, from 1990 to 2008. All cases had H&E stains available for review and paraffin blocks for immunohistochemical staining. The cases had been previously characterized using an extensive panel of antibodies for B and T cells by flow cytometric and immunohistochemical staining techniques. Flow cytometric studies performed on our 34 B-ALL cases suggested that only 6% of the patients with B-ALL had pro-B-ALL (CD34+/CD19+/CD10−), 76% had common ALL (CD34+/CD19+/CD10+), and 18% had a pre-B-ALL immunophenotype (CD34+ -CD19+/CD10+ ). Our flow cytometric panel did not include other specific markers (terminal deoxynucleotidyl transferase, CD22, CD20, and cytoplasmic μ chain) that would be useful to confirm the stage of differentiation of our B-ALL cases. The H&E-stained slides and other ancillary studies were reviewed, and the diagnosis was agreed upon using the morphologic and immunohistologic criteria defined by the World Health Organization classification.

**Tissue Microarray Constructs**

H&E-stained slides from all 83 cases were reviewed to localize precisely the target areas of the tumor in corresponding paraffin-embedded tissue blocks for inclusion within the tissue microarray. The tissue microarray was made using a manually operated instrument that uses 2 separate needles for punching the donor and recipient blocks (Beecher Instruments, Silver Spring, MD). Tissue cylinders (1 mm in diameter) retrieved from the donor blocks were oriented within the recipient block using the instrument’s X-Y micrometer adjustment knobs. Two cores from each donor block were punched and oriented into the recipient blocks. The completed recipient block was incubated at 37°C for 20 minutes to facilitate embedding of the tissue cores in paraffin.

**Immunohistochemical Staining**

Sections of the tissue microarray blocks were mounted onto positively charged slides, baked at 60°C for 60 minutes, deparaffinized in xylene, rehydrated in graded alcohols, and rinsed. For heat-induced epitope retrieval and staining for PAX5, OCT-2, BOB.1, CD20, CD79a, and MUM1, the tissue sections were placed in a pressure cooker containing 10 mmol/L citrate buffer, pH 6.0. For PU.1 and CD22 staining, the tissue slides were placed in a pressure cooker containing EDTA solution, pH 8.0. Pressure cookers were then heated in a microwave (1,500 W) for 12 minutes. After cooling the sections for 20 minutes at room temperature, endogenous peroxidase activity was blocked using 3% hydrogen peroxide in distilled water, and for the prevention of nonspecific background staining, blocking serum was applied (Santa Cruz staining kit, Santa Cruz Biotechnology, Santa Cruz, CA). The sections were then incubated with the monoclonal antibodies to PU.1 (clone G148-74, dilution 1:50; Pharmingen, San Diego, CA), PAX5 (clone 24/PAX5, dilution 1:200; BD Transduction Laboratories, Lexington, KY), CD20 (clone L26, dilution 1:600; DakoCytomation, Glostrup, Denmark), CD79a (clone JCB117, dilution 1:300; DakoCytomation, Glostrup), and MUM1 (clone MUM1p, dilution 1:500; DakoCytomation, Glostrup) and polyclonal antibodies to CD22 (dilution 1:100; Abcam, Cambridge, MA), OCT-2 (dilution 1:1,000; Santa Cruz Biotechnology), and BOB.1 (dilution 1:500; Santa Cruz Biotechnology) for 30 to 60 minutes at room temperature. For the detection of bound primary antibodies, the EnVision technique (DakoCytomation, Carpinteria, CA) with diaminobenzidine as a substrate was used. Sections of a tonsil with reactive lymphoid hyperplasia served as positive and negative controls. The following staining patterns were considered positive: nuclear expression for MUM1, PU.1, OCT-2, and PAX5; cytoplasmic expression for CD20 and CD79a; cytoplasmic and/or membranous expression for CD22; and nuclear and/or cytoplasmic staining for BOB.1. Because the nuclear counterstain appeared to be strong for OCT2 and MUM1 in B-ALL cases, we examined slides without counterstains to evaluate for possible weak nuclear staining. A positive stain was interpreted as greater than 10% of the neoplastic cells staining. Two authors (M.R.N. and S.S.) interpreted the staining results independently. Any disagreement in interpreting the results was resolved by consensus.

**Results**

The results of immunohistochemical staining in B-ALL [Image 1], Burkitt lymphomas [Image 2], and T-ALL [Image 3] are summarized in [Table 1]. All B-ALLs (Image 1B) and Burkitt lymphomas (Image 2B) were positive for PAX5 while none of the T-ALL cases were positive. Nuclear staining with PAX5 was strong and homogeneous. In all cases of B-ALL and Burkitt lymphoma, more than 50% of the cells showed positive staining. Overall, PAX5 was 100% sensitive and specific for B-lineage neoplasms.

Blasts cells from most B-ALL cases (29 of 34) coexpressed CD79a (Image 1C) and PAX5. The staining pattern of CD79a was cytoplasmic, and reactivity was strong in most cases. A few cases (5 of 34) did not coexpress the 2 antigens.
**Image 1** B-acute lymphoblastic leukemia/lymphoma. **A**, H&E staining (×1,000). **B**, Strong nuclear staining for PAX5 (×1,000). **C**, Cytoplasmic positivity for CD79a (×1,000). **D**, Cytoplasmic and membrane positivity for CD22 (×1,000). **E**, Nuclear staining for PU.1 (×1,000). **F**, Cytoplasmic and nuclear positivity for BOB.1 (×1,000).
Image 2I Burkitt lymphoma. A, H&E staining (×1,000). B, Strong nuclear staining for PAX5 (×1,000). C, Cytoplasmic positivity for CD79a (×1,000). D, Cytoplasmic and membrane positivity for CD22 (×1,000). E, Nuclear staining for PU.1 (×1,000). F, Cytoplasmic and nuclear positivity for BOB.1 (×1,000).
and of those, all were PAX5+/CD79a−. Nonneoplastic mature B cells expressed CD79a in all these cases, which served as an internal positive control. No T-ALL cases expressed CD79a; however, 29 of 30 cases of Burkitt lymphoma were CD79a+(Image 2C). The specificity of CD79a for B-cell lineage was 100%. The sensitivity values were 79% and 97% for B-ALLs and Burkitt lymphomas, respectively.

CD22 positivity was seen in 27 of 34 B-ALLs (Image 1D) and 14 of 30 Burkitt lymphomas (Image 2D), resulting in a sensitivity of 79% and 47%, respectively. Immunoreactivity for CD22 was mostly diffuse, strong, and cytoplasmic. None of the 19 T-ALLs expressed CD22.

Nuclear PU.1 expression was seen in 33 of 34 B-ALLs (Image 1E) and in 23 of 30 Burkitt lymphomas (Image 2E). BOB.1 expression was invariably nuclear and seen in 32 of 34 B-ALLs (Image 1F) and in 27 of 30 Burkitt lymphomas (Image 2F). Unlike PAX5, BOB.1 and PU.1 showed weak to moderate nuclear positivity in a variable percentage of leukemic cells: in 12 (63%) of 19 (Image 3B) and 15 (79%) of 19 (Image 3C) of T-ALL cases, respectively.

Nuclear expression of OCT-2 was identified in 9 (26%) of 34 B-ALL cases. OCT-2 expression was present in 23

Table 1
Immunohistochemical Expression of Pan–B-Cell Markers and B-Cell Transcription Factors in B/T-Acute Lymphoblastic Leukemia/Lymphoma and Burkitt Lymphoma

<table>
<thead>
<tr>
<th>Markers</th>
<th>B-Acute Lymphoblastic Leukemia/Lymphoma (n = 34)</th>
<th>Burkitt Lymphoma (n = 30)</th>
<th>T-Acute Lymphoblastic Leukemia/Lymphoma (n = 19)</th>
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</thead>
<tbody>
<tr>
<td>PAX5</td>
<td>34 (100)</td>
<td>30 (100)</td>
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</tr>
<tr>
<td>BOB.1</td>
<td>32 (94)</td>
<td>27 (90)</td>
<td>12 (63)</td>
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<tr>
<td>PU.1</td>
<td>33 (97)</td>
<td>23 (77)</td>
<td>15 (79)</td>
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<td>CD79a</td>
<td>29 (85)</td>
<td>29 (97)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>CD22</td>
<td>27 (79)</td>
<td>14 (47)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>CD20</td>
<td>2 (6)</td>
<td>30 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>OCT-2</td>
<td>9 (26)</td>
<td>23 (77)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>MUM1</td>
<td>3 (9)</td>
<td>5 (17)</td>
<td>0 (0)</td>
</tr>
</tbody>
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PAX5 and shows that robust staining for PAX5 is present in all B-ALL cases that lack or show equivocal staining for other pan-B-cell markers.

The process of proliferation and differentiation of stem cells into mature B cells requires different lineage-determination genes. A number of transcription factors have an important role in this process. The PAX5 gene is a member of the paired box (PAX) family of transcription factors and is required to maintain the identity and function of B cells during early and late B-cell development. PAX5, the B cell–specific activator protein (BSAP), is expressed in pro-B, pre-B, and mature B cells, but not in plasma cells, and its expression suppresses the expression of genes that orchestrate differentiation along other lineages. Several previous studies have discussed the potential value of PAX5 in the diagnosis of a wide range of B-cell lymphoid tumors. Our findings of high sensitivity and specificity of PAX5 in

Discussion

Although the number of antibodies for use in the immunostaining of paraffin-embedded tissue has greatly expanded and immunohistochemical analysis is considered a useful ancillary technique in the diagnosis of B-ALL, controversy exists regarding the best marker or combination of markers to assign B-cell lineage in ALL. Our study confirms the usefulness of PAX5 and shows that robust staining for PAX5 is present in all B-ALL cases that lack or show equivocal staining for other pan-B-cell markers.

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(77%) of 30 Burkitt lymphomas (Image 2G). Nuclear staining of OCT-2 was weak to strong and patchy, and staining intensity varied greatly among positive nuclei. CD20 was expressed in all 30 Burkitt lymphomas (Image 2H), but in only 2 of 34 B-ALLs. MUM1 stained few cases of Burkitt lymphomas (5/30; Image 2I). Weak nuclear expression of MUM1 was seen in 3 (9%) of 34 B-ALL cases. All cases of T-ALL were negative for CD20, OCT-2, and MUM1.

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the diagnosis of B-ALL confirm previous studies that have found this antibody to be diagnostically valuable. Falini and Mason\(^1\) commented on the usefulness of PAX5 in the diagnosis of B-lymphoblastic neoplasms in paraffin-embedded biopsy specimens. Tiacci et al\(^1\) reported that all 150 cases of B-ALL were positive for PAX5; however, PAX5 was not detected in 50 cases of T-ALL. Similarly, Torlakovic et al\(^1\) reported that PAX5 was also expressed in all 20 B-ALL cases studied but was not seen in T-ALL. One study reported PAX5 positivity in 4 of 11 T-ALL cases; however, this study was small and the results were not reproduced in further studies.\(^1\) Recently, Tzankov et al\(^1\) reported that PAX5 expression was seen rarely in peripheral T-cell lymphoma.

Some cases of acute myeloid leukemias may demonstrate aberrant expression of PAX5, leading to a false impression of B-cell lineage.\(^1\) PAX5 expression has been associated most commonly with the t(8;21)(q22;q22) in acute myelogenous leukemia and occasionally with promyelocytic leukemia.\(^5\) It has been reported that most of the cases with t(8;21) also are positive for CD19.\(^1\) However, blasts in acute myelogenous leukemia usually have characteristic morphologic, cytochemical, immunophenotypic, and molecular features, which make it easy to separate acute myelogenous leukemia from B-ALL. Aberrant PAX5 expression has also been reported in few cases of small, round blue cell tumors, including neuroendocrine carcinomas, Merkel cell carcinoma, small cell carcinoma, alveolar rhabdomyosarcoma, and medulloblastoma.\(^1\) In such a setting, however, PAX5 staining should be used as part of a panel of immunohistochemical stains as an adjunct to morphologic assessment.

We found that PAX5 is a more sensitive marker than CD79a for lineage assignment in B-cell ALL. During B-cell development, cytoplasmic expression of CD79a/CD79b begins at the pro-B cell stage.\(^2\) In our study, coexpression of PAX5 and CD79a was found in 85% of B-ALLs and also in 97% of Burkitt lymphomas. Chu et al\(^1\) reported PAX5 expression in all 29 CD20– cases of B-ALL, whereas 23 (79%) of 29 cases expressed CD79a. Although not supported by our study, others have found CD79 expression in about 40% of T-ALLs.\(^2\) The absence of aberrant coexpression of CD79a in T-ALL cases in our study compared with others may be explained by the relatively low number of cases we studied.

The CD79a promoter gene is regulated by PAX5 and partly regulated by PU.1. PU.1 is a member of the ETS family of transcription factors and is crucial for the early stages of B-cell development. It is reported that PU.1 expression is seen in a subset of T-ALLs.\(^1\) Hence, it was suggested that the expression of CD79a in T-ALL is related to PU.1 expression.\(^1\) Our study demonstrated that the vast majority of cases of B-ALLs expressed PU.1; however, 15 of the 19 cases of T-ALL showed definite positivity for PU.1. It is interesting that 12 of these 15 cases were also positive for BOB.1. Chu et al\(^1\) demonstrated that BOB.1 is preferentially expressed in B-ALL but not in T-ALL and suggested that it could be of diagnostic value. Our findings suggest that BOB.1 is not a reliable marker for B-ALL. The discrepancy in BOB.1 specificity may be attributed to the use of polyclonal antibodies in our study as opposed to Chu et al,\(^1\) who used a monoclonal antibody. Expression of PU.1 and BOB.1 in T-lineage lymphoid neoplasms has also been reported.\(^4\)

OCT-2 protein is a B-cell–specific transcriptional coactivator and is expressed in the earliest stage of B-cell development. Although OCT-2 was fairly specific in our study, a large proportion of B-ALL cases (74%) were negative. The majority of our Burkitt lymphoma cases (77%) were positive. While not seen in our cases, some studies have shown that OCT-2 may be expressed at low levels in some T-cell lymphomas.\(^5\)

CD20 is the most commonly used immunohistochemical marker for B-lineage assignment and is helpful for mature B-cell neoplasms; however, fewer than 20% of B-ALLs are positive for CD20. Unlike B-ALL, all cases of Burkitt lymphomas express CD20. Rare case reports documented aberrant CD20 expression in T-cell lymphomas.\(^3\) However, all our T-ALLs were negative for CD20. Similar to CD20, the transcription factor MUM1 was positive in fewer than 10% of B-ALL cases and negative in all T-ALL cases. In addition, only 5 cases of Burkitt lymphoma expressed MUM1. The findings in this study confirm that CD20 and MUM1 are not reliable immunohistochemical markers for the diagnosis of immature B-cell neoplasms because both markers lack sensitivity.

CD22 is a B-lymphoid lineage–specific molecule expressed in the cytoplasm of pro-B and pre-B cells and on the surface of more mature B cells.\(^2\) Previous studies demonstrated that the majority of B-ALL cases expressed cytoplasmic and weak-to-moderate levels of surface CD22.\(^2\) Our results agree with prior observations reporting that CD22 is B-lineage restricted.\(^2\) In addition, our observation confirms the reliability of CD22 immunostaining in the diagnosis of B-ALL. A large portion of our Burkitt lymphoma cases (53%) were CD22–. It is interesting that by flow cytometric immunophenotyping, most B-ALLs and Burkitt lymphomas are dim to moderately CD22+ (surface or cytoplasmic). This discrepancy may be due to greater sensitivity of flow cytometric compared with immunohistochemical analysis.

We have found that PAX5 is a useful surrogate marker that can be used to establish the B-cell lineage of rare cases of CD79a– and CD22– B-ALL. Although lineage determination and proper diagnosis of B-ALL are usually performed by flow cytometry, PAX5 immunohistochemical staining may provide valuable information in cases in which a cellular bone marrow aspirate cannot be obtained owing to marrow fibrosis or marked hemodilution. The expression of PU.1 and BOB.1 in both B- and T-ALL makes these markers less useful in the
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differential diagnosis of these entities. Although it is reported that OCT-2 is an early B-cell differentiation antigen, only 26% of our B-ALL cases were positive.

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References


