B-Cell Transcription Factor Expression and Immunoglobulin Gene Rearrangement Frequency in Acute Myeloid Leukemia With t(8;21)(q22;q22)

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ABSTRACT

Objectives: To assess a large series of patients with acute myeloid leukemia (AML) with t(8;21) for both IGH@ and IGK@ B-cell gene rearrangements and for expression of PAX5, OCT2, and Bob.1 by immunohistochemistry and expression of CD19, CD79a, CD20, and CD22 by flow cytometry immunophenotyping.

Methods: A total of 48 cases of AML with t(8;21)(q22;q22) were evaluated by immunohistochemistry and/or heavy chain and light chain immunoglobulin rearrangement studies where paraffin-embedded and/or fresh frozen material was available for study; previously performed flow cytometry studies were also reviewed in available cases.

Results: Our study yielded 1 of 19 cases of AML with t(8;21) with an IGH@ gene rearrangement; blasts were associated with weak PAX5 expression. In addition, expression of antigens CD79a by flow cytometry and OCT2 by immunohistochemistry were highly associated with PAX5 expression, and CD19 was expressed in most cases assessed.

Conclusions: Although B-cell antigen and B-cell transcription factor expression is seen in the majority of AMLs with t(8;21)(q22;q22) and correlates with PAX5 expression, immunoglobulin gene rearrangements are an uncommon event in this group of leukemias.

Acute myeloid leukemia with t(8;21)(q22;q22); RUNXI-RUNXIT1 [AML with t(8;21)] accounts for approximately 5% of all AMLs.1 It results from a translocation from the RUNX1 gene (also known as AML1), located on chromosome 21, with the RUNXIT1 gene (previously known as ETO), located on chromosome 8. This leukemia is classified within the AMLs with recurrent cytogenetic abnormalities and confers a favorable prognosis as compared with other AML types when treated with high-dose cytarabine. AML with t(8;21) is generally more common in younger patients and accounts for 10% to 15% of cases of AML in children.1,3 Several characteristics of AML with t(8;21) may be noted on initial morphologic and flow cytometric examination, the latter of which may include expression of CD19, CD79a, and CD56.4,7
PAX5, a transcription factor involved in the development of B cells, is also expressed in testicular tissue and neural tissue. Like other members of the PAX family of transcription factors for their respective sites, PAX5 plays a key role in early B-cell development from hematopoietic precursors and is expressed throughout B-cell development, with diminished to no expression as the mature B cell reaches the plasma cell stage of development. The PAX5 gene transcript is also referred to as a B-cell activator protein and regulates expression of genes involved in B-cell proliferation, activation, and differentiation, including CD19, BLK, and Igμ/CD79a. PAX5 is also associated with the downregulation of other lineage-specific markers, including NOTCH1, GATA1, and M-CSF-R, which serve to restrict cell development to within the B-cell lineage.

Recent studies have examined the expression of PAX5 and other B-cell–associated markers in AML, including AML with t(8;21). Several groups have noted CD19 expression in a high percentage of AML with t(8;21), with frequencies ranging from 75% to 93% of cases as assessed by flow cytometry. Cytoplasmic CD79a expression has also been reported in cases of AML with t(8;21), albeit a smaller percentage of cases relative to CD19.

A study by Tiacci et al. was one of the first to assess the expression of PAX5 by immunohistochemistry and noted that 15 (35.7%) of 42 cases of AML with t(8;21) were positive for PAX5 expression; in this study, PAX5 expression was limited to cases of AML with t(8;21) and not seen in AMLs with other recurrent cytogenetic abnormalities. PAX5 messenger RNA transcript expression also was demonstrated by Western blot in all 8 cases tested, 4 of which were negative by immunohistochemistry. Valbuena et al. examined 28 cases of AML with t(8;21) and demonstrated expression of PAX5 in all cases; additional immunohistochemistry revealed a subset of cases (12/16, 75%) positive for the expression of OCT2, a transcription factor expressed in mature B cells. Similarly, Gibson et al. examined 16 cases of AML with t(8;21) and noted expression of PAX5 by immunohistochemistry in 7 (44%), 3 (19%), and 6 (38%) cases of AML with t(8;21), as well as OCT2 and Bob.1, another transcription factor expressed in mature B cells.

In a few in vitro murine studies, PAX5 has been noted to bind to the conserved promoter regions of the activating genes RAG1 and RAG2, which are involved in gene rearrangement of the VH to D or J loci for production of immunoglobulin. Although the frequency of PAX5 expression and other B-cell marker expression is fairly well characterized, to our knowledge, no monoclonal immunoglobulin gene rearrangements have been reported in AML with t(8;21). Oster et al. examined 69 cases of AML for immunoglobulin heavy chain gene rearrangements by restriction endonuclease digestion, which were present in 10 cases. Six of these 10 cases expressed CD19. Most cases with a heavy chain gene rearrangement represented AML-M1 or AML-M4 leukemia (using the French-American-British [FAB] classification), with 1 categorized as FAB AML-M2. No corresponding karyotypic studies were performed. In the study by Valbuena et al., 3 cases of AML with t(8;21) were assessed for IGH@ and IGK@ rearrangements, and all were negative. Kita et al. tested 15 cases of AML with t(8;21), and all cases showed germline immunoglobulin genes without rearrangement.

**Materials and Methods**

The files of the Department of Pathology at Stanford University Medical Center were retrospectively searched for cases of AML with t(8;21)(q22;q22) from 1989 through 2011. All experiments performed were approved by our institutional review board. Forty-eight cases with either a bone marrow aspirate and/or a bone marrow trephine biopsy specimen were available for review, and the diagnosis was confirmed after examination of morphology/histology, flow cytometry, and cyogenetic studies. All cases reviewed demonstrated the RUNX1-RUNX1T1 fusion gene on concurrent fluorescent in situ hybridization studies or the t(8;21) translocation on karyotype studies. Thirty-four of the 48 cases of AML with t(8;21) pulled represented the original diagnostic bone marrow core biopsy sample or aspirate, with 29 original diagnostic bone marrow trephine cores available. Overall, 40 total bone marrow trephine cores were assessed by immunohistochemistry. All bone marrow biopsy specimens were fixed in Bouin fixative and decalcified in Formical-4 (Decal Chemical, Tallman, NY) prior to paraffin embedding. Flow cytometric data were available for 36 of the 48 cases of AML with t(8;21), and 19 cases of AML with t(8;21) had frozen tissue available for immunoglobulin gene rearrangement studies.

Immunohistochemistry was performed using automated immunohistochemical stainers (Benchmark XL; Ventana Medical Systems, Tucson, AZ) with antibodies to PAX5 (clone 24, dilution 1:25; BD Biosciences, San Jose, CA), OCT2 (polyclonal, dilution 1:500; Santa Cruz Biotechnology, Santa Cruz, CA), and Bob.1 (polyclonal, dilution 1:500; Santa Cruz Biotechnology); positive control lymph node and normal bone marrow tissue were stained concurrently and simultaneously assessed. Cases were scored by 2 pathologists (R.C.J. and T.I.G.). PAX5, OCT2, and Bob.1 were assessed on a 0 to 2+ scale, where 0 indicates no evidence of nuclear staining; 1+, weak positive nuclear staining in a subset of blasts; and 2+, strong positive nuclear staining similar in intensity to the positive control.
mature B cells. A numerical percentage was also ascribed to the subset of positive blasts when cases were positive.

Flow cytometry used standard antigens to B cells, natural killer/T cells, and myeloid cells and was performed on a FACSCanto or FACSCalibur flow cytometer platform (Becton Dickinson, San Jose, CA). Antigens to CD19, CD20, CD22, and CD79a were judged partially positive if 10% to 19% of the blasts showed expression and strongly positive when 20% or more of the blasts were positive relative to the isotype control.

Immunoglobulin gene rearrangement studies were performed on all frozen bone marrow aspirate material available for study. Genomic DNA was isolated by using the QIAGEN (Louisville, KY) DNeasy Blood & Tissue Kit according to the manufacturer’s instructions. The IGH and IGK Gene Clonality Assays (InVivoScribe Technologies, San Diego, CA) were used to identify clonal B-cell immunoglobulin heavy chain (IGH@) and light chain (IGK@) gene rearrangements. All 5 master mix sets (FR1, FR2, FR3, DH, and JH primers) and both master mix sets (Vk, Jk, Jk-Ck intron, and Kde primers) of fluorescence capillary detection primers covering the V-J regions of the immunoglobulin gene were used in separate amplification reactions per the IgH and IgK clonality assays, respectively, as developed by InVivoScribe and recommended by the BIOMED-2 Concerted Action report.31 The amplicon product sizes were determined using the ABI 3100 platform (Applied Biosystems, Foster City, CA) and analyzed using GeneMapper software (Applied Biosystems). When DNA primers amplified a region, a clonal population of cells yielded 1 or 2 prominent amplicons within the required size range. Such findings were interpreted as evidence of a clonal gene rearrangement. All positive results were confirmed by demonstrating an identical-sized amplicon on a repeat assay. A positive and negative control was performed for each run; DNA quality was established individually for each patient specimen via a specimen control–sized ladder that was required to show amplification products of at least 400 base pairs.

Results

By immunohistochemistry, 30 (75%) of 40 cases of AML with t(8;21) demonstrated expression of PAX5 in blasts, with a smaller subset demonstrating OCT2 expression Table 1 and Image 1. Most cases (24/40, 60%) showed 1+ expression of PAX5 in blasts, with the percentage of PAX5 staining in blasts ranging from 5% to 40%. Six of 40 cases showed 2+ expression, which ranged from 10% to 60% of blasts. OCT2 expression was noted in a smaller subset of cases. Of the 40 cases of AML stained for OCT2, 6 (15%) showed expression of OCT2, and all cases showed 1+ expression. All 6 cases with OCT2 expression expressed PAX5 by immunohistochemistry, with 3 cases showing 1+ PAX5 intensity and 3 cases showing 2+ PAX5 intensity in myeloblasts. Bob.1 expression was not noted in any of the 40 cases assessed.

Regarding flow cytometric marker expression, 29 (81%) of 36 cases showed expression of CD19. Twenty-one (58%) of 36 cases had CD19 expression in greater than 20% of the blast population, with the remainder (8/36, 22%) regarded as partially positive for CD19. The percentage of blasts with partial positive or positive expression ranged from 11% to 96% overall (mean, 42%). CD79a was expressed in 6 (24%) of 25 cases of AML with t(8;21), with expression ranging from 11% to 45% of blasts (mean, 25%). Two of the 6 cases showed expression in greater than 20% of blasts; both of these cases lacked CD19 expression. Of the remaining cases with partial positive expression of CD79a, 3 showed strong expression and 1 showed partial positive expression of CD19. Three (75%) of 4 cases and 4 (100%) of 4 cases that demonstrated CD79a expression by flow cytometry showed OCT2 expression and PAX5 expression by immunohistochemistry, respectively. None of our cases expressed CD20 or CD22. Nineteen cases with t(8;21) demonstrated expression of PAX5 in blasts, with the percentage of PAX5 staining in blasts ranging from 5% to 40%. Six of 40 cases showed 2+ expression, which ranged from 10% to 60% of blasts. OCT2 expression was noted in a smaller subset demonstrating weak 1+ PAX5 expression in 20% of the myeloblasts and was negative for OCT2 expression. Since this case was one of residual acute leukemia obtained during induction.

### Table 1

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<tr>
<th>Flow Cytometry, Immunohistochemistry, and Immunoglobulin Gene Rearrangement Results in Acute Myeloid Leukemia With t(8;21)*</th>
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<tr>
<td><strong>Total Cases (n = 48)</strong></td>
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<td><strong>Flow Cytometry (n = 36)</strong></td>
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<tr>
<td>% Cases with ≥ partial positive expression</td>
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<tr>
<td>% Positive with PAX5 coexpression</td>
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<tr>
<td>% Positive with OCT2 coexpression</td>
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<td><strong>Immunoglobulin Gene Rearrangement Studies (n = 19)</strong></td>
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<td><strong>IGH@ and IGK@</strong></td>
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*Values are presented as number/total number assessed (%).

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chemotherapy (the original diagnosis of acute leukemia was made on a peripheral blood specimen), concomitant flow cytometric assessment was not performed on this sample. No cases demonstrated an IGH@ rearrangement.

A review of this patient’s complete medical record, including pathology and laboratory studies at our institution, yielded no history of either a B-cell or a plasma cell neoplastic process. The original diagnosis of leukemia was made on a peripheral blood specimen, and marrow studies obtained at week 3 and week 6 of induction chemotherapy showing residual or recurrent leukemia were evaluated for a concurrent B-cell neoplasm. No obvious mature B-cell infiltrate was appreciated on immunohistochemistry of the bone marrow core at week 3 or week 6, and the week 3 induction chemotherapy specimen showed scattered plasma cells with polytypic expression of light chains (data not shown). Serum protein electrophoresis studies were never performed in this patient.

**Image 1** B-cell marker expression in acute myeloid leukemia with t(8;21)(q22;q22). A, Case with weak (1+) PAX5 nuclear expression by immunohistochemistry; lymphocytes with strong expression are in the background (×600). B, Another case with moderate to strong (2+) PAX5 expression by immunohistochemistry (×600). C, Same case as B with weak (1+) OCT2 expression within a subset of blasts (×600). D, Flow cytometric findings of the same case as B revealing strong expression of CD19 and partial cCD79a expression. MPO, myeloperoxidase; SSC, side scatter.
Discussion

As normal B cells undergo development from the precursor marrow stage to the pro-B-cell stage, PAX5 expression plays a central role in B-cell marker expression and function. On developing B cells, CD19 is one of the first B-cell-specific markers acquired in the pro-B-cell stage, closely followed by CD79a, with later expression of CD20 and CD22 prior to the mature B-cell stage. Heavy chain immunoglobulin rearrangements, initiated by recombination factors including RAG1 and RAG2, also occur during the late

**Image 2** B-cell phenotype in single case of acute myeloid leukemia (AML) with t(8;21)(q22;q22) possessing an IGH@ rearrangement. A, Core biopsy showing sheets of immature mononuclear cells consistent with blasts. Cytogenetic studies showed a t(8;21)(q22;q22) translocation confirming residual AML (H&E; ×600). B, Immunohistochemistry shows mild (1+) nuclear expression of PAX5 in myeloblasts (×600). C, Myeloblasts lack OCT2 and Bob.1 expression (×600). D, Capillary electrophoresis results from the IgH gene clonality assay revealing a solitary peak of appropriate amplicon length (D\(_{17-48}\)) consistent with a reproducible monoclonal IGH@ gene rearrangement (control ladder peaks of ~200 base pairs and ~400 base pairs are also present). No rearrangement of the IGK@ gene was identified (data not shown).
pre–B-cell stage, and subsequent light chain rearrangements are completed by the immature B-cell stage. Many of these processes are under the regulation of the transcription factor PAX5. PAX5 expression, present in normal B lymphocytes and many B-cell lymphomas, is not specific for B lineage, and its expression is noted in AML, especially AML with t(8;21). Seventy-five percent of our cases demonstrated expression of PAX5. This frequency is similar to the percentage of cases positive in previous studies, which ranged from 35% to 100%. Although Western blot appears to be a more sensitive technique for asserting PAX5 expression in AML with t(8;21), we did not perform such studies.

By immunohistochemistry, however, we noted a lower frequency of cases with OCT2 and Bob.1 expression as compared with Valbuena et al and Gibson et al, respectively. This may be due to the loss of antigenicity from our Bouin fixation and decalcification processes; in addition, long-term storage of our paraffin-embedded tissues may have affected antigen integrity. We did obtain similar flow cytometric frequency of cases positive for CD19, CD79a, CD20, and CD22 expression relative to other studies.

Strong CD19 expression was noted in 58% of our cases, and 81% of our cases demonstrated at least partial expression. A smaller subset (24%) of our cases also demonstrated CD79a expression, which has been reported with similar frequency in the literature. Furthermore, no expression of CD20 and CD22 was noted, similar to other studies assessing for those markers.

All cases with partial CD79a expression demonstrated partial positive or positive expression of CD19, and 2 cases had CD79a expression in 25% and 45% of the leukemic blasts by flow cytometry. It is important to note, however, that these cases should not be regarded as meeting criteria for mixed-phenotype acute leukemia (B/myeloid). Per the most recent agreed-upon nomenclature for B-lymphoid/myeloid biphenotypic acute leukemia according to the European Group for the Immunological Characterization of Leukemias and the 2008 World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues, leukemias with adequate myeloperoxidase expression and strong CD19 expression (>20% blasts) must also express either CD22 or CD79a in more than 20% of the leukemic blasts, or, if CD19 is weakly expressed (10%-20%), 2 of either CD79a, CD20, and/or CD10 must be strongly expressed.

None of our cases met established criteria for biphenotypic or mixed-phenotype acute leukemia. In addition, the WHO classification would not consider cases with AML with t(8;21) to fall into the mixed-phenotype acute leukemia category even if the criteria were met because of the specific clinical features of AML with t(8;21).

Acute leukemias can often present at an extramedullary location, and these cases are often challenging, requiring a battery of immunohistochemical stains to establish lineage since flow cytometric studies often are not ordered initially. The differential diagnosis of a large cell hematolymphoid neoplasm includes both lymphoblastic leukemias and AMLs as well as lymphomas such as diffuse large B-cell lymphoma, anaplastic large cell lymphoma, blastic mantle cell lymphoma, and peripheral T-cell lymphomas, not otherwise specified. Thus, a positive PAX5 and/or OCT2 immunohistochemical result must be interpreted with caution, even if a positive concomitant clonal immunoglobulin gene rearrangement is also detected.

The significance of the presence of an immunoglobulin gene rearrangement in 1 case of our series of AML with t(8;21) is unknown. Although we postulate that the IGH@ rearrangement may be related to the leukemic clone, ultimately the possibility of the presence of an underlying clonal B-cell process cannot be excluded entirely, although no obvious mature B-cell infiltrate was appreciated on PAX5, OCT2, or Bob.1 immunohistochemistry of the bone marrow core at week 3 and week 6 of induction chemotherapy.

As normal B cells mature through the pro–B-cell stage, productive rearrangements of one of the alleles of the V-D-J complex are required for cells to survive to the pre–B-cell stage. Similar successful rearrangements are required of the V-J complex for B cells to survive to the immature B-cell stage as well. The transcription factor PAX5 is expressed throughout most stages of B-cell maturation and is thought to coordinate downstream transcription effects in the cell. Knockout mice deficient in PAX5 were notable for a lack of germinal center formation in secondary lymphoid tissues and, more important, lacked expression of surface immunoglobulin-positive B cells and serum immunoglobulin production.

PAX5 knockout mice have also been noted to have markedly decreased levels of the VH-DJH recombination of the IGH@ gene locus as compared with wild-type mice. One may postulate that the relative overexpression of PAX5 by leukemic cells may similarly orchestrate downstream activation of other transcription factors as well as DJH and VH-DJH recombinations. It is uncertain whether other cooperating transcription factors required for normal B-cell differentiation, including EBF1, Ets1, and E2A, are expressed in AML with t(8;21) and whether this may affect the relative infrequency of immunoglobulin gene rearrangements seen in this study. Additional studies assessing other transcription factors in AML with t(8;21) may help to shed additional light on this process.

The exact mechanism underlying aberrant cross-lineage antigen expression of CD19 and cCD79a in AML with t(8;21) is not completely understood but is suggested to be related to PAX5 expression. Furthermore, cross-lineage antigen expression is not exclusive to AML with t(8;21) and is often reported in other acute leukemias, including expression of myeloid antigens CD117, CD13, and CD33 in T- and B-lymphoblastic leukemias.
transcriptional factor expression is also not exclusive to AML with t(8;21). Recently, NOTCH1 mutations in a subset of cases of AML with aberrant CD7 expression have been described.45 Similarly, a subset of acute promyelocytic leukemias have been shown to express T-cell–associated transcripts, including cytoplasmic CD3, as well as demonstrate T-cell receptor (TRG@) gene rearrangements.46

Two similar hypotheses regarding the mechanisms underlying these presumed aberrant genomic changes and expression profiles are termed lineage infidelity and lineage promiscuity. Lineage infidelity proposes that somatic genetic aberrations that occur during leukemogenesis result in an abnormal and potentially unexpected phenotype. Lineage promiscuity similarly suggests that the leukemic clone itself is an expansion of a progenitor line with putative dual or multiple lineage potential.44 It is possible that the presence of the IGH@ rearrangement noted in one of our cases of AML with t(8;21) may be related to one of these proposed processes independent of or in conjunction with PAX5 expression. Further studies, including those examining the presence or absence of immunoglobulin transcripts in AML, including AML with t(8;21), may be necessary to support or refute our findings. On the basis of the findings of our study, we believe that immunoglobulin rearrangements in AML with t(8;21) are an uncommon occurrence.

Conclusion

To our knowledge, this is the largest study to date assessing the presence of immunoglobulin gene rearrangements in AML with t(8;21). Expression of CD19 and PAX5 occurred in most cases, and CD79a and OCT2 expression was noted in a subset of cases. These findings are similar to B-cell marker expression reported in previous studies. In addition, we found 1 case out of 19 positive for an IGH@ rearrangement, which heretofore has not been described in the literature to our knowledge. This case also showed weak PAX5 expression but did not show either OCT2 or Bob.1 expression. Further studies are needed to establish the true frequency of immunoglobulin gene rearrangements in AML with t(8;21).

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References


