Precursor B-Lymphoblastic Transformation of Grade I Follicle Center Lymphoma

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Abstract

Part of the natural history of follicle center lymphoma (FCL) is transformation to a more aggressive neoplasm, almost always a diffuse large B-cell lymphoma. We describe a rare example of a precursor B-lymphoblastic transformation of grade I FCL occurring in a 45-year-old woman 12 years after initial presentation and 3 years after successful treatment for a diffuse large cell transformation. The lymphoblastic lymphoma shared the same immunoglobulin heavy chain gene rearrangement as the FCL as assessed by polymerase chain reaction amplification and direct sequencing, as well as identical kappa light chain gene rearrangements by Southern blot analysis. The immunoglobulin heavy chain variable gene sequences of both tumors showed numerous identical base substitutions compared with germline sequences and 3 additional mutations in the lymphoblastic lymphoma not present in the low-grade FCL. These results indicate origin of the lymphoblastic process from the mature follicle center B-cell clone, rather than divergent origin of the 2 tumors from a common immature B-cell precursor.

The typical clinical course of low-grade follicular lymphomas (follicle center lymphomas [FCLs], grades I and II) is indolent behavior with good response to therapy but with multiple relapses over a period of many years. An additional important aspect of the natural history of these lesions is transformation to a more aggressive diffuse subtype of lymphoma. Estimates of the overall frequency of such transformation generally range from 25% to 35%. However, the actuarial risk of histologic transformation is much higher, around 60% to 70% at 10 years. The most common histologic appearance of transformed lymphoma is diffuse large cell lymphoma, although occasional transformations to small noncleaved cell lymphomas also are observed. These transformations historically have carried a poor prognosis, although good responses may be obtained with intensive chemotherapy in some patients. Alterations of the p53 gene, and less commonly the c-myc gene, have been implicated in the histologic progression in some cases.

Only very rare transformations of FCL to precursor B-lymphoblastic lymphoma have been described; the c-myc gene rearrangement has been implicated in the reported cases. We report the case of a woman with a 10-year history of follicular low-grade lymphoma in whom precursor B-lymphoblastic lymphoma developed after she had been treated successfully for a diffuse large cell transformation several years earlier. Direct sequence analysis of the immunoglobulin heavy chain gene (IgH) variable-diversity-joining (VH, DJH) rearrangements documented clonal identity between the low-grade FCL and the lymphoblastic lymphoma.
**Case Report**

A 33-year-old woman sought care in 1985 because of lymphadenopathy. Biopsy of an inguinal lymph node in August 1985 revealed malignant lymphoma, follicular, predominantly small cleaved cell type (FCL, grade I). At the time, she was treated only with local radiation therapy. The patient was followed up until October 1986, when a submandibular lymph node biopsy specimen revealed low-grade follicular lymphoma. Again she was treated with radiation therapy to the neck and upper chest.

She did well until 1994, when biopsy of a left femoral lymph node revealed diffuse large B-cell lymphoma. She was treated with 8 cycles of cyclophosphamide, doxorubicin, vincristine, and prednisone (also known as CHOP) through January 1995.

In October 1995, she sought care because of right axillary lymphadenopathy. Biopsy revealed persistence of low-grade follicular lymphoma. She was observed without treatment and was well until March 1997, when she experienced rapid enlargement of a right axillary mass. Biopsy revealed precursor B-lymphoblastic lymphoma. A bone marrow examination at that time revealed involvement by low-grade lymphoma but no evidence of a lymphoblastic process. She was then treated with vincristine, doxorubicin, asparaginase, prednisone, cyclophosphamide, cytarabine, and mercaptopurine, resulting in complete remission. This was followed by consolidative radiation therapy to the right axilla.

In July 1997, a paravertebral mass developed that was considered on clinical grounds to be relapse of the lymphoblastic lymphoma. Treatment at that time included local radiation, as well as etoposide, cisplatin, and cytarabine through August 1997. Her subsequent course was complicated by neutropenic fever. A matched unrelated donor was found, and she was admitted to the hospital in October 1997 for matched unrelated donor bone marrow transplantation. The posttransplant course was complicated by pulmonary edema and an adult respiratory distress syndrome–like picture, as well as by sepsis and occipital hemorrhage, and the patient died in December 1997.

**Materials and Methods**

Pathologic material was available for review from the 1985, 1986, 1994, 1995, and 1997 specimens; paraffin blocks were available for additional studies on the 3 most recent biopsy specimens. Frozen tissue was available for the 1995 and 1997 biopsies. The lymph node specimens were fixed in B-5, 10% neutral buffered formalin, or both. The 3 most recent biopsy specimens were embedded in paraffin by routine methods, sectioned, and stained with H&E. Immunohistochemistry was performed according to a standard, previously described avidin-biotin-peroxidase technique. The antibodies used, along with dilutions and pretreatment regimens, are given in Table I.
Southern Blot Analysis for IgH

This analysis was performed using a 1,020-base-pair probe, IGJ6 (DAKO, Carpinteria, CA) hybridized to BgII and BamH/HindIII digests. Southern blot analysis for the immunoglobulin kappa chain gene was performed using a 540-base-pair probe, IGK5 (DAKO) hybridized to BgII and HindIII digests. Both probes are supplied with fluorescein labels for nonradioactive detection using the DAKO chemiluminescent system for nucleic acid blotting (DAKO). Southern blot analysis for c-myc rearrangement was performed as previously described.25

Polymerase Chain Reaction Analysis for bcl-2 Gene

DNA was extracted from the frozen tissue samples using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN). The extracted DNA was used as a template for amplification of the t(14;18)-specific fusion of the bcl-2 locus and the IgH locus. The bcl-2 primers targeted the major breakpoint region, and the 3’-minor cluster region. Polymerase chain reaction (PCR) was performed using a nested assay according to a previously published method.26

PCR Amplification of the IgH Gene

DNA from the 1995 FCL was extracted from previously frozen tissue as described in the preceding section. DNA from the 1997 lymphoblastic lymphoma was extracted from paraffin-embedded tissue, as previously described.27 The IgH genes were amplified in a seminested PCR method using primers to the framework region (FR) II28 or III29 of the IgH and primers to the IgH JH region.29 The PCR was performed in a total reaction volume of 50 µL containing 20 pmol of each outer primer, a 50-mmol/L concentration of potassium chloride, a 10-mmol/L concentration of tris(hydroxymethyl)-aminomethane hydrochloride (pH 8.3), a 1.5-mmol/L concentration of magnesium chloride, with 2.5 U of Taq polymerase (Amplitaq, Perkin-Elmer, Branchburg, NJ), 200-µmol/L concentrations of each deoxynucleoside triphosphate (Perkin-Elmer), and 10 µL of template DNA. The first round of PCR consisted of 30 cycles followed by a 1:1,000 dilution and a second round of 20 cycles. The PCR conditions were as follows: 94 C for 15 seconds, 55 C for 30 seconds, and 72 C for 10 seconds. Each PCR round was preceded by a 10-minute denaturation at 95 C and followed by a final extension of 20 minutes at 72 C. Ten microliters of PCR product was analyzed by electrophoresis using a precast 5% polyacrylamide gel (Bio-Rad, Hercules, CA). The electrophoresed products were visualized under UV light after ethidium bromide staining.

Sequencing of PCR Products

The PCR products were purified using the High Pure PCR Product Purification kit (Roche Molecular Biochemicals, Indianapolis, IN) and directly sequenced using the BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) and an automated sequencer (ABI Prism 310 Genetic Analyzer, Applied Biosystems). PCR products were sequenced in both directions. Sequences were compared with the GenBank data library and V BASE (V BASE Sequence Directory, Ian M. Tomlinson, Samuel C. Williams, Olga Ignatovich, et al, MRC Centre for Protein Engineering, Cambridge, England) using Sequencher software (Gene Codes Corporation, Ann Arbor, MI).

Results

Morphologic Features and Immunohistochemistry

The original inguinal lymph node from 1985 and the 1986 submandibular relapse demonstrated effacement of normal nodal architecture by a closely packed proliferation of fairly uniform follicular structures with absent or severely attenuated mantle zones. The predominant cells within the follicular structures were small cleaved cells with elongated, twisted, or angulated nuclei with condensed chromatin and absent, nucleoli. Occasional large noncleaved cells were scattered within the follicles. Tingible body macrophages were almost absent, and mitotic figures were infrequent.

The left femoral lymph node from 1994 demonstrated a diffuse proliferation with prominent vascularity, sclerotic

![Image 11](https://example.com/image11.jpg) Low-power view of diagnostic inguinal lymph node biopsy from 1985 demonstrates a closely packed proliferation of uniform follicular structures with attenuated mantle zones, consistent with follicle center lymphoma (H&E, original magnification x100). The nodules were composed predominantly of small cleaved cells.
bands, and areas of coagulative necrosis. The vascular component produced the appearance of a nesting architecture in some areas, but no true nodularity was discerned. In some areas, the infiltrate was composed of a mixture of small and large cells. Although many of the small cells were reactive CD3+ T cells, a substantial population of CD20+ cells with small cleaved cell morphologic features also was present. The large cells expressed CD20 and had oval or irregular nuclei with finely distributed chromatin and 1 to several distinct eosinophilic nucleoli and moderate amounts of eosinophilic cytoplasm. In some areas of the lymph node, the infiltrate was composed almost exclusively of the large cell component. Mitotic activity was brisk, and scattered apoptotic debris was present. The areas composed entirely of large cells warranted a diagnosis of diffuse large cell lymphoma, apparently arising in a lesion with diffuse mixed-cell morphologic features. Additional immunohistochemical analysis demonstrated bcl-2 protein expression by the small and large B cells; p53 was positive in the nuclei of rare neoplastic cells (<1%).

The right axillary lymph node from 1995 revealed histologic features similar to those noted in the original 1984 biopsy specimen, except that the neoplastic follicles were less regular and not as densely packed. The cellular composition of the follicles again was composed predominantly of small cleaved cells. The neoplastic cells again expressed bcl-2 but failed to express p53.

The 1997 right axillary lymph node demonstrated complete effacement of lymph node architecture by a diffuse, monomorphous, neoplastic infiltrate that extended into surrounding fat. The cells were small to medium in size with round or slightly irregular nuclei, very fine chromatin, generally inconspicuous nucleoli, and scant cytoplasm. Mitotic activity was brisk, but little apoptotic debris was present. Anti-CD20 highlighted scattered cells that were clearly part of the neoplastic infiltrate, although the large majority were negative. The majority of the cells were positive for CD79a, bcl-2, terminal deoxynucleotidyl transferase, and CD43 but failed to react with anti-myeloperoxidase, CD68, and lysozyme. No follicular lymphoma or large cell lymphoma was present in this biopsy specimen.

Molecular Studies

Southern blot analysis for IgH and immunoglobulin kappa chain gene yielded identical results for the 1995 low-grade FCL and the 1997 lymphoblastic lymphoma. Two rearranged bands were detected in each of the 2 digests probed for IgH. One rearranged band was detected in the BglII digests probed for immunoglobulin kappa chain gene; the HindIII digests probed for immunoglobulin kappa chain gene were in the germline configuration. No rearrangements of the c-myc gene were detected in either specimen.

IgH PCR with the FRIII seminested protocol yielded clonal bands of identical size in the 1995 and 1997 specimens. However, the band in the 1995 specimen was extremely weak and did not allow for direct sequencing. Subsequent analysis with an FRII primer again yielded...
clonal bands of identical size in both specimens **Image 5**. Direct sequence analysis of these PCR products revealed clonal identity between the 1995 low-grade FCL and the 1997 lymphoblastic lymphoma, with both tumors using V\(_H\)3-48 and J\(_H\)6. Both the 1995 and 1997 specimens showed numerous base substitutions compared with published germline sequences; these substitutions were identical in the 2 specimens with the exception of 3 additional point mutations in the 1997 lymphoblastic lymphoma that were not present in the 1995 FCL (Image 5). PCR for the t(14;18) rearrangement was negative in both cases.

**Discussion**

We describe a very unusual precursor B-lymphoblastic transformation of low-grade FCL. Although aggressive transformations of FCLs are well described, they generally take the form of diffuse large B-cell lymphomas or, occasionally, small noncleaved cell lymphomas.\(^3\,^5\,^{11}\) Interestingly, our patient experienced lymphoblastic transformation after successful treatment for a large cell transformation and subsequent relapse with the low-grade tumor. To our knowledge, only 4 precursor B-lymphoblastic transformations of FCL have been described in the English-language literature.\(^{11}\,^{21}\,^{23}\) One of
these also had earlier undergone a large cell transformation.22 The time at diagnosis of the transformed lymphoma ranged from simultaneously with the low-grade FCL to 8 years after initial lymphoma diagnosis. All 4 cases were described as having typical precursor lymphoblastic cytomorphologic features. In 2 cases, the precursor B-cell nature of the transformed lesion was established definitively.22,23 In the third case, the cells were described as being terminal deoxynucleotidyl transferase–positive, but the cell lineage was not established,11 and in the fourth case, the diagnosis was made on the basis of cellular morphologic features.21 The 3 patients for whom clinical follow-up was reported all died within 9 months of documentation of the lymphoblastic process, as in the present case.11,21,23

Approximately 85% of FCLs have rearrangements of the \( \text{bcl-2} \) gene into the immunoglobulin heavy chain joining region.30-34 While both the FCL and the lymphoblastic lymphoma in the present case overexpressed \( \text{bcl-2} \) protein, PCR analysis of the major breakpoint region and 3’-minor cluster region regions of the \( \text{bcl-2} \) gene failed to reveal rearrangements in either tumor. Although it is possible that our analysis was negative for technical reasons, we think this was unlikely as the DNA was good quality and the analysis was repeated with the same results in a second independent laboratory (data not shown). Therefore, this FCL lacked \( \text{bcl-2} \) gene rearrangement or had an unusual breakpoint outside the usual major breakpoint region and 3’-minor cluster region regions. Unfortunately, conventional cytogenetic analysis was not performed.

The underlying pathogenetic mechanisms of transformation of FCL have been the subject of several studies. The \( p53 \) gene is the genetic locus that has been found to be most commonly involved in FCL transformation.15,18 Sander et al18 found that \( p53 \) mutations were associated with transformation in 25% to 30% of transformed FCLs. In their hands, immunohistochemical demonstration of \( p53 \) protein overexpression correlated well with the presence of mutation. They were able to demonstrate rare \( p53 \)-positive cells in the preceding low-grade component of the transformed lymphoma, but large numbers of cells overexpressing the protein were restricted to the more aggressive tumors. Sander et al18 postulated that these rare cells in the low-grade component represent the earliest evidence of the \( p53 \) mutated clone, which subsequently expands and is susceptible to...
additional genetic events. In fact, in 1 case, they were able to document progressive accumulation of p53-positive cells in sequential biopsy specimens before transformation. In the present case, no p53 expression was documented in the low-grade tumor, and the higher grade lesions demonstrated only rare positive nuclei.

Mutations in the c-myc gene have been documented in 8% of transformed follicular lymphomas. Interestingly, translocations involving 8q24 have been present [in addition to (t;14;18)] in all 4 cases of precursor B-lymphoblastic transformation of FCL [t(8;14) in 3 cases and t(8;22) in 1 case], rearrangements usually associated with Burkitt lymphoma. Although we were unable to document c-myc rearrangement in our case by Southern hybridization using a probe to c-myc exon 1, this method may be relatively insensitive for detecting c-myc rearrangements.

Among the previous reports, only De Jong et al established clonal identity between the 2 tumor components on the basis of similarly sized rearranged bands on Southern blot analysis of the immunoglobulin heavy chain joining region and bcl-2. Clonal identity of the remaining cases was inferred on the basis of the presence of a t(14;18) in the lymphoblastic component. In the present case, sequence analysis of the variable regions of the IgH genes amplified from the low-grade FCL and the precursor B-lymphoblastic lymphoma revealed the same V\_H\_DJ\_H rearrangement in both tumors, indicating that they are clonally related. Southern blot analysis of the IgH and kappa light chain genes also revealed clonally rearranged bands of identical size. Such a relationship potentially could result from common origin from a B-cell precursor clone that already had rearranged its immunoglobulin genes or from clonal evolution of the mature B-cell tumor resulting in “dedifferentiation” to a lymphoblastic stage of B-cell development. Analysis of the sequence data derived from these tumors sheds light on this question. Previous analyses have shown that follicular lymphoma cells are derived from germinal center B cells that have undergone somatic mutation of their immunoglobulin genes and that they continue to accumulate mutations after malignant transformation. The variable sequences of both tumors in the present case contained numerous base changes compared with germline sequences. These changes were identical in the FCL and lymphoblastic lymphoma, with the exception of 3 additional mutations found in the lymphoblastic but not the FCL component. These data provide strong evidence that the lymphoblastic lymphoma in this case indeed arose from the mature follicle center B-cell clone rather than from an immature B-cell precursor.

References


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