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Acquisition of a t(11;14)(q13;q32) in clonal evolution in a follicular lymphoma with a t(14;18)(q32;q21) and t(3;22)(q27;q11.2)

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Chromosome translocations involving an immunoglobulin (*IG*) locus and another gene, either *BCL* or *MYC*, are common events in B-cell lymphoma. Occasionally, two *IG* loci, one with *BCL* and the other with *MYC*, are simultaneously involved; such cases are classified as double-hit (DH) lymphomas. These tumors often show intermediate histologic features between those of diffuse large B-cell lymphoma and those of Burkitt lymphoma. Patients with DH lymphoma have a poor prognosis. Rarely, lymphomas in which three *IG* loci are simultaneously involved with two different *BCL* genes and *MYC* have been reported. These cases are classified as triple-hit lymphomas; virtually all these are aggressive tumors with an even worse prognosis. We present here a unique case of follicular lymphoma (FL) with rearranged *BCL2*, *BCL6*, and *BCL1* (also known as *CCND1*) genes. Lymphoma cells at first clinical relapse had a complex karyotype that included a t(3;22)(q27;q11) and t(14;18)(q32;q21). About 15 years after initial diagnosis, the lymphoma cells showed clonal cytogenetic evolution and acquired a t(11;14)(q13;q32). This article is the first case report of a low grade B-cell lymphoma that had three lymphoma-associated reciprocal translocations not involving *MYC* and that had a long indolent clinical course.

Keywords B-cell lymphoma, double translocation, triple translocation, clonal evolution

Deregulation of dominant acting oncogenes through chromosome translocations is considered one of the early pathogenetic events in B-cell lymphomagenesis. In most cases, the oncogene is activated by its juxtaposition to the regulatory region of *IG* genes, primarily the heavy chain gene at 14q32. In some cases, the oncogene is activated through somatic mutations within the regulatory region of the oncogene or through juxtaposition to the regulatory region of a non-*IG* gene (1–3).

Since the identification of the B-cell leukemia lymphoma gene 1 (*BCL1,* also known as *CCND1*) (4), several *BCL* genes have been identified by cloning the translocation junction between the *IG* genes and the partner chromosomes. A few of these, namely *CCND1*, *BCL2*, *and BCL6*, are frequently recurrent and are characteristically associated with a specific histologic type of lymphoma. However, this association is not exclusive. Tumors with multiple specific translocations occurring concurrently have long been reported (5,6). Two translocations, one involving a *BCL* gene and the other involving *MYC* have been reported in 3–14% of diffuse large B-cell lymphomas (DLBCLs); these tumors are called DH lymphomas (7–12). Some of these DH lymphomas are de novo tumors. In other tumors, the *MYC* translocation is a secondary event that occurs during tumor progression. These tumors have histologic, immunophenotypic, and genetic features that are intermediate, between those of DLBCL and those of Burkitt lymphoma (BL); therefore, they are considered B-cell lymphomas, unclassifiable, with features between DLBCL and BL (13,14). Clinically, the prognosis of patients with DH lymphoma is poor with short survival (7,9,10,15–19). Lymphoma involving *MYC* along with two different *BCL* (20–27), or three different *BCL* genes (28) has been reported; in one study, the frequency of the triple-hit (TH) lymphoma was 1.02% (12). Survival of patients with TH lymphoma was even worse than that of those with DH lymphoma (26,29); nonetheless, occasionally a TH tumor

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with FL histology has been reported (30). To our knowledge, no nonaggressive lymphoma with three reciprocal translocations not involving *MYC* has been previously reported; here, we present a case of follicular lymphoma with three translocations not involving *MYC*.

Patient and methods

Clinical case

A 55-year-old African American man presented with lymphadenopathy in August of 1995. He was diagnosed with follicular small cleaved cell lymphoma involving an axillary lymph node (LN), bone marrow (BM), and peripheral blood (PB). Flow cytometry of the lymphoma cells demonstrated the following immunophenotypic features: CD5(−), CD10(−), CD11c(−), CD19(+), CD20(+), CD22(+), CD23(+), CD38(dim+), FMC-7(+), kappa immunoglobulin light chain (+), and lambda immunoglobulin light chain (−). The patient was treated with an unknown regimen of chemotherapy. In September of 1995, a fine-needle aspirate of another axillary LN revealed persistent disease with a small proportion of larger neoplastic cells in addition to the small cleaved cells. Flow cytometry analysis of this specimen demonstrated a similar immunophenotype but with partial expression of CD10. These specimens were not evaluated for karyotype. The further clinical course was uneventful. Restaging scans in December 2008 showed a few mildly enlarged LNs on both sides of the diaphragm (right axillary LNs 9 and 11 mm, left para-aortic LNs up to 12 mm, right external iliac LNs up to 11 mm, bilateral inguinal LNs up to 12 mm). At that time, the cytological results of an LN sample were positive for a CD10(+) B-lineage lymphoma. An excisional biopsy of a left groin LN in February 2009 confirmed this to be follicular lymphoma, grade I with focal grade II lesions. This lymphoma showed clonal karyotypic changes. BM was negative for involvement, and the disease was considered stage IIIA.

The patient was followed clinically and continued to do well with mild inguinal lymphadenopathy, normal blood counts, no B symptoms, and stable CT results. In March of 2010, recurrent lymphoma was identified in the PB with 9% lymphoma cells. By May of 2010, PB lymphoma cells increased to 42%. Clinically, the patient continued to do well with no B symptoms, no palpable lymphadenopathy, and no hepatosplenomegaly. In September 2010, the patient developed mild thrombocytopenia and the lactate dehydrogenase (LDH) level increased to 232 IU/L. BM and PB were involved by lymphoma. Clinically, the patient was still doing well but was lost for further follow-up at this institution before the initiation of therapeutic intervention.

Histology, immunohistochemistry, and flow cytometry

Surgically obtained tissue specimens were processed for standard histopathologic evaluation after routine H&E staining and immunohistochemical staining. Immunophenotypic analysis was performed on BM, PB, or LN samples, following the standard protocols for flow cytometric analysis. Detailed immunophenotypic analysis was performed using a four-color antibody panel. The antibodies and clones (from Pharmingen, Becton Dickinson, Franklin Lakes, NJ, unless otherwise stated) included CD2(S5.2), CD3(SK7), CD4(SK3), CD5(L17F12), CD7(4H9), CD8(SK1), CD10(W8E7), CD19(SJ2C1), CD20(L27), CD23(EBVCS-5), CD30(Ber-H2; DAKO, Carpentaria, CA), CD34(8G12), CD38(HB7), CD45(2D1), CD56(NCAM16.2), CD71(L01.1), CD79a(HM47), CD79b(HM47), FMC-7(FMC7), monoclonal κ(TB28-2), monoclonal λ (1−15), polyclonal κ (2) (Beckman Coulter, Brea, CA), polyclonal λ (1) (Beckman Coulter), HLA-DR(L243), TdT (TdT-6) (Thermo Fisher Scientific, Waltham, MA) and appropriate isotypic control antibodies (X39 and X0). A standard staining protocol was used, as described elsewhere (31). Data were collected using a BD FACScalbur flow cytometer instrument with BD CellQuest Pro software. Data were then analyzed using BD Paint-A-Gate software through cluster analysis, which involves identifying biologically distinct populations of cells that cluster differentially with respect to six measured parameters: forward and orthogonal light scatter properties and the fluorescence properties in each of the four channels of four-color flow cytometry.

Chromosome analysis and fluorescence in-situ hybridization study

Lymphocytes prepared by mincing the fresh LN tissue, and whole BM were cultured for 24 hours in RPMI1640 supplemented with 10% FBS, 1% L-glutamine, and 1% Pen-strep. These short-term cultures were harvested following standard protocols. Air-dried metaphase spreads were trypsin-G-banded, and karyotypes were described following ISCN 2009 (32). The fluorescence in situ hybridization (FISH) probes used in this study—*CCND1*, *BCL2*, *BCL6*, and *MYC*—were purchased from Abbott Molecular (Des Plains, IL); these probes were used following the standard FISH protocol (codenaturation of the probe and target at 74°C for 4 min, hybridization overnight at 37°C, washing at 72°C for 2 min). Images were captured using ASI software (Applied Spectral Imaging, Chicago, IL).

Results

Histology, immunohistochemistry, and flow cytometry

Original diagnostic material from 1995 was not available for re-evaluation. Histologic results of the February 2009 excisional biopsy of the left inguinal LN showed typical features of follicular lymphoma, including a nodular infiltrate with crowded nodules containing predominantly small centrocytes and scattered large centroblasts (less than five per high-power field (HPF)); occasional nodules had 5 to 15 large centroblasts per HPF and occasional mitotic figures. Immunohistochemically, the neoplastic cells were CD20(+), CD10(+), BCL2(+), Ki-67(+) (approximately 30% of cells), CD3(−), MUM-1(−), and cyclin D1(−). Flow cytometry showed similar immunophenotypic features. Although the proliferation rate was slightly increased, overall the findings best fit follicular lymphoma, grade I with focal grade II