Is relapse of mantle cell lymphoma (MCL) preceded by an “in situ” pattern in an otherwise reactive lymph node in a patient with history of MCL?

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Introduction

Mantle cell lymphoma (MCL) is a B cell neoplasm with a relatively aggressive clinical course and a poor outcome. Immunophenotypically, almost all cases express both cyclin D1 and BCL2. CD5 and CD43 are expressed in most cases [1, 2]. The t(11;14)(q13;q32) translocation is considered the primary genetic event in the pathogenesis of MCL. This translocation causes aberrant overexpression of cyclin D1, a positive regulator of the cell cycle [3–5]. By fluorescence in situ hybridization (FISH) analysis, t(11;14) is present in virtually all MCL cases [6]. There are also non-random secondary chromosomal aberrations in MCL. Trisomy 12 has been reported in 25% of cases. Tetraploid clones are

more commonly detected in the pleomorphic and blastoid variants than in the classical type (80%, 36%, and 8%, respectively). Cases of MCL demonstrating t(8;14)(q24;q32) are rare, but associated with more aggressive behavior [5]. MCLs with chromosomal alterations involving 3q27 (BCL6) have been described and those cases demonstrated increased BCL6 protein expression [7].

Recently, SOX11, a neural transcription factor has been reported as a specific marker for both cyclin D1-positive and negative MCL. Besides MCL, SOX11 is also expressed in lymphoblastic leukemia/lymphoma and Burkitt lymphoma [8]. Patients with SOX11-negative MCL exhibit more frequent nonnodal presentations, predominantly hypermutated IGVH, and lack of genomic complexity with improved survival compared to patients with SOX11-positive MCL [9, 10].

There are occasional cases showing pathological involvement almost exclusively restricted to the mantle zone of the follicles (“in situ” MCL) in an otherwise reactive lymph node without disruption of normal architecture [1, 3, 4, 11, 12]. It is not certain whether these in situ MCL cases represent an early-stage event in the development or an indolent form of MCL. Furthermore, the clinicopathologic significance of focal involvement of a lymph node by an in situ pattern MCL in a patient with a past history of MCL has not been described in the literature.

Case report

A 62-year-old female presented in June 2008 with significant cervical and supraclavicular lymphadenopathy. An excisional biopsy of a cervical lymph node showed a classical MCL. FISH analysis revealed t(11;14). The patient was classified as Ann Arbor stage

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IV MCL with bone marrow involvement. Additional workup including serologies for HIV and hepatitis B and C was negative. She was treated with 8 cycles of hyper-Cyclophosphamide, Vincristine, Adriamycin, Dexamethasone, and methotrexate and cytarabine and had a complete response to chemotherapy. Posttreatment bone marrow biopsy showed no morphologic and molecular evidence of residual lymphoma.

Approximately 2 years later, the patient was seen for an interval follow-up. She stated that she may have felt enlarging lymph nodes in her neck, but she was otherwise feeling well. She denied any fevers, chills, night sweats, or weight loss. A complete review of systems was negative at that time. CT scans showed interval enlargement of multiple cervical and axillary lymph nodes. An excisional cervical lymph node biopsy revealed reactive follicular hyperplasia. Because of concern that the patient may have experienced a clinical relapse despite the initial histologic findings demonstrated in the excised lymph node, cyclin D1 immunohistochemistry was completed on the tissue and revealed foci of an in situ pattern MCL. However, FISH analysis for t(11;14) was negative. Since the patient was asymptomatic at that time, an approach of watchful waiting was taken and a bone marrow biopsy was not performed.

Within 5 months after being diagnosed with in situ pattern MCL, the patient presented with fevers and night sweats of several weeks duration. CT scans showed

increased size and number of left axillary lymph nodes, worrisome for progression of disease. Excisional biopsy of an axillary lymph node revealed frank recurrent MCL. A bone marrow biopsy was negative. The patient subsequently underwent 4 cycles of chemotherapy for recurrent disease. Of note, there has been no peripheral blood involvement by flow cytometric analysis.

Materials and methods

The Leica Microsystems Bond-III immunostainer (Bannockburn, IL, USA) was used for immunohistochemical staining using Bond Polymer Refine Detection kit, (Bannockburn, IL, USA) for the following antibodies: CD3, CD5, CD10, CD20, CD23, CD43, cyclin D1, BCL2, BCL6, MIB1, SOX11, and p27. For SOX11 antibody, Sigma Prestige (St Louis, MO, USA), a 1:50 dilution was used utilizing the Bond retrieval solution, ER2 for 20 min. The antibody was incubated for 15 min and normal tonsil was used for a positive control. For anti p27 antibody, BD Transduction Laboratories (San Diego, CA, USA), a 1:200 dilution was used utilizing Bond retrieval solution, ER1 for 20 min. The antibody was incubated for 15 min and normal prostate was used as a positive control.

FISH was performed according to a protocol recommended by the manufacturer, using a kit for t(11;14), t

Fig. 1 Follicular hyperplasia with in situ pattern mantle cell lymphoma. **a** Reactive follicular hyperplasia (hematoxylin-eosin, $\times 10$). **b** Thin and unexpanded neoplastic mantle zone (hematoxylin-eosin, $\times 40$). **c** Cyclin

D1-positive neoplastic cells within the mantle zone ($\times 40$). **d** Aberrant co-expression of CD5 with weak staining intensity ($\times 40$). **e** BCL2 expression ($\times 40$)

(8;14), BCL6, and CEP12 (Vysis). Four to 6- μ m thick paraffin sections were placed on positively charged slides. Next, the slides were air dried, followed by the addition of the probe mixture (7 μ L LSI/WCP hybridization buffer, 1 μ L probe, and 2 μ L purified water). Specimens were coverslipped and sealed using rubber cement. The slides were placed in a Hybrite (Vysis) with melt temperature/time of 73°C/6 min and a hybridization/temperature/time of 37°C/4 h/overnight. Slides were soaked in 2X SSC/0.3%NP-40 to remove the coverslip, then immerse slides in 2.0X SSC/0.3%NP-40 at 73°C for 2 min. The slides were air dried and then counterstained with 10 μ L 4,6-dimidino-2-phenylidone/antifade (Vysis).

Results

The first lymph node of 2008 showed effaced nodal architecture with a distinct mantle zone growth pattern. The atypical lymphocytes were small to intermediate sized with irregular nuclear outlines. They were positive for cyclin D1, CD5 (weak), and BCL2. A low proliferation rate (<10%) was demonstrated by Ki-67. Flow cytometry detected a monoclonal, lambda light chain restricted B cell population expressing CD19, CD20, CD5, and FMC7 without the expression of CD10. t(11;14) was detected by FISH analysis in 96% of all cells.

The second lymph node of 2010 revealed reactive follicular hyperplasia characterized by multiple polarized germinal centers with numerous tingible body macrophages (Fig. 1a). The mantle zones of these follicles were morphologically unremarkable (Fig. 1b). However, cyclin D1 immunohistochemistry revealed a thin rim of cyclin D1-positive neoplastic cells within the mantle zone with no or very little spread into the interfollicular areas (Fig. 1c). This in situ pattern MCL with cyclin D1 expression in the mantle zone was focal and present only in a few follicles. The in situ pattern was also highlighted by the weak expression of CD5 (Fig. 1d). BCL2 showed strong staining intensity (Fig. 1e). Overall, the number and localization of cyclin D1-positive cells were almost identical to those of weak CD5, BCL2, and CD43-positive mantle cells. Confirmatory FISH analysis for t(11;14) was attempted on the touch prep slides that showed negative findings. This may have indicated a false-negative result due to the sampling issue regarding the very low numbers of the tumor cells in the tissue to be meaningfully analyzed by the FISH technique.

The third lymph node of 2010, obtained 5 months after the second lymph node revealed frank recurrent MCL. There was a diffuse proliferation of neoplastic lymphocytes with features of a classical type (Fig. 2a). There was no evidence of blastoid or pleomorphic variants. Persistent

weak expression of CD5 was observed (Fig. 2c). Increased proliferation rate (30–40%) was also noted. SOX11 and p27 were diffusely positive (>90%). The tumor cells were negative for CD10 and CD23 but weakly positive for BCL6 protein in small numbers.

The detection of cyclin D1 expression with the absence of t(11;14) in the in situ pattern MCL led the authors to investigate further molecular abnormalities retrospectively, including break-a-part rearrangement of 3q27, t(8;14) and trisomy 12. The first lymph node was negative for all of the above abnormalities, except for t(11;14) being solely positive. Although the in situ pattern MCL was negative for t(11;14), it demonstrated tetraploidy of 3q27 and CEP12 in 12% each of all the cells. However, no t(8;14) translocation was identified in the in situ pattern MCL. Interestingly, the recurrent MCL showed complex cytogenetic abnormalities including t(11;14) in 92%, tetraploidy of CEP12 in 86%, tetraploidy of 3q27 (BCL6) in 95%, and extra copies of MYC, IGH, and CEP8 with no t(8;14) fusion signals in 98% in all cells analyzed (Fig. 3a–d).

Discussion

This is the first reported case demonstrating the morphologic, immunophenotypic, and genotypic findings as well as the clinical significance of in situ pattern MCL in a patient with a prior history of frank MCL. In this case, disease progression with evidence for clonal evolution was also identified from the original MCL (2008) to the recurrent lymphoma (2010).

From the immunohistochemical point of view, cyclin D1 was essential to recognize the in situ pattern, since the cytologic abnormality or expansion of the mantle zone was not identified on the H&E stain. Although cyclin D1 was particularly helpful to assess the diagnosis of in situ pattern MCL, the aberrant co-expression of CD5 and CD43 was of high diagnostic value. In addition, persistent weak expression of CD5 was notable throughout the original, in situ pattern and recurrent overt MCL. In our case, diffuse nuclear positivity of SOX11 was observed in both the original and recurrent MCL. However, the in situ pattern MCL did not demonstrate SOX11 expression.

Although the clinical significance of in situ pattern MCL in an asymptomatic patient with a history of prior MCL is not yet fully known, our case suggests that overt relapse of MCL may be preceded by an in situ pattern MCL in an otherwise reactive lymph node. This indicates management with watchful waiting is essential to detect early overt disease in a patient with a history of MCL diagnosed with intercurrent in situ pattern MCL.

Fig. 2 Recurrent overt mantle cell lymphoma. **a** Diffuse proliferation of monomorphic lymphocytes with irregular nuclear contours and inconspicuous nucleoli (hematoxylin-eosin, $\times 60$). **b** Cyclin D1 ($\times 40$). **c** Persistent weak expression of CD5 ($\times 40$). **d** BCL2 ($\times 40$). **e** SOX11 ($\times 40$)

Fig. 3 Fluorescence in situ hybridization analysis of recurrent mantle cell lymphoma. **a** Detection of $t(11;14)(q13;q32)$; cells with dual fusion (arrows showing yellow pattern) along with single green and orange signals. **b** Tetraploidy of CEP12. **c** Tetraploidy of 3q27 (BCL6) with no break-a-part pattern. **d** FISH for $t(8;14)(q24;q32)$ with extra copies of MYC, IGH, and CEP8 with no indication of a significant fusion pattern

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Conflict of interest The authors declare that they have no conflict of interest.

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