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Signet-ring cell lymphoma: clinicopathologic, immunohistochemical, and fluorescence in situ hybridization studies of 7 cases

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ABSTRACT

Context: Signet-ring cell lymphoma (SRCL) is a rare morphologic variant of non–Hodgkin lymphoma. Although it was initially reported as a rare morphologic variant of follicular lymphoma (FL), SRCL has to date been described in most types of non–Hodgkin lymphoma, mostly as single-case reports.
Objective: To study SRCL systematically by immunohistochemical stains and fluorescent in situ hybridization analyses.
Design: Seven SRCL cases were stained for CD3, CD5, CD20, PAX-5, CD10, CD21, CD23, cyclin D1, BCL2, BCL6, Ki-67, and MUM-1, and were analyzed by fluorescent in situ hybridization for *BCL2, BCL6, MYC*, and *MALT1* rearrangements. Clinical information and patient outcome were reviewed in all patients.
Results: The patients were 3 women and 3 men, ranging in age from 31 to 75 years (average 60.3 years). The lesions involved lymph nodes, tonsil, parotid gland, soft tissue, and breast. There were 4 FLs, 1 diffuse large B-cell lymphoma (DLBCL), 1 DLBCL with FL, and 1 DLBCL with marginal zone lymphoma. All cases had typical signet-ring cell morphology. They were positive for CD20 and BCL-2, and had low-to-intermediate Ki-67 proliferation index (10%–40%) except in the parotid DLBCL with FL (70%). BCL-6 was detected in all but 1 FL (6/7). Fluorescent in situ hybridization detected *IGH/BCL2* translocation in 1 FL, increased *BCL6* copy number in another FL, *BCL6* rearrangement, and increased copy number of *MYC* and *MALT1* in the DLBCL with marginal zone lymphoma.
Conclusions: The FL with signet-ring cell morphology (1/5) tends to lack *IGH/BCL2* translocation, and an extended immunohistochemical study is recommended for correct diagnosis and classification of SRCL.

1. Introduction

Signet-ring cell lymphoma (SRCL) is a rare morphologic variant of non–Hodgkin lymphoma (NHL). The lymphoma cells typically contain abundant cytoplasmic inclusions and the nuclei may be displaced to the periphery of the cells. Although it was initially reported as a rare morphologic variant of follicular lymphoma (FL), SRCL has to date been described in most types of NHL, mostly as single-case reports and including both cutaneous and systemic NHL, such as FL, small lymphocytic lymphoma, lymphoplasmacytic lymphoma, marginal zone lymphoma of mucosa-associated lymphoid tissue, diffuse large B-cell lymphoma (DLBCL), T-cell lymphoma and anaplastic large cell lymphoma, and plasma cell myeloma [1]. Signet-ring cell lymphoma commonly involves lymph nodes, but has also been reported in extranodal tissues including the skin, gastrointestinal tract, salivary gland, breast, central nervous system, and bone marrow [1-16]. It may pose a diagnostic challenge or even be misdiagnosed, especially in the absence of an extensive immunohistochemical (IHC) study and flow cytometric analysis due to a limited needle biopsy sample [6,17].

We report 7 cases of SRCL from 6 patients with detailed clinicopathologic studies including extensive IHC study and fluorescence in situ hybridization (FISH) analyses for gene rearrangements involving *BCL2*, *BCL6*, *MYC*, and *MALT1*. Our study demonstrated that an extensive IHC study is preferred for adequate diagnosis and classification of SRCL.

2. Materials and methods

Our pathology department archives from 1988 to 2016 for "signet ring cell lymphoma, lymphoma with signet ring cells" were searched. Clinical information and the results of flow cytometric analysis were collected from the medical records. Five cases from 4 patients were identified. Two additional cases were contributed by the collaborating

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colleagues. This study was approved by the institutional review board of our institute.

Immunohistochemical stains were performed at the referring institutions or in the laboratory at Indiana University Health Pathology Laboratory using the prediluted, ready-to-use antibodies (CD3, clone IR503; CD20, IR604; CD10, IR648; BCL2, IR614; BCL6, IR625; Ki-67, IR626; MUM-1, IR644) from Dako (Santa Clara, CA) with a Dako Autostainer Plus instrument following the manufacturer's protocols [18].

Fluorescence in situ hybridization studies were performed using 4-μm tumor tissue sections that were mounted on positively charged glass slides. An accompanying hematoxylin and eosin slide was reviewed to determine tumor location. Slides were baked at 65°C ± 5°C for 1 hour, deparaffinized in xylene at room temperature, and washed in 100% ethanol and air-dried. Pretreatment included exposure to 0.2 N HCl, followed by 1 M sodium thiocyanate and pepsin at 37°C to digest proteins in the tissue sample. After pepsin treatment, slides were viewed with phase microscopy to ensure adequate digestion of the tumor tissue. Denaturation, ethanol dehydration, and hybridization with the LSI MYC Dual Color, Break Apart Rearrangement; LSI IGH/MYC, CEP 8 Tri-color Dual Fusion Translocation; LSI BCL6 Break Apart and LSI IGH/BCL2 Dual Color, Dual Fusion Translocation (Abbott Molecular, Des Plaines, IL) probes were carried out as per manufacturer's instructions. Cells were counterstained with DAPI and observed under a Leica DMRXA2 fluorescence microscope. Two hundred cells were analyzed by 2 readers (100 cells apiece) for each of the 3 probes. Scoring criteria included the selection of single nuclei with representation of at least 1 signal for each color/probe/nucleus.

3. Results

3.1. Clinical features

A total of 7 cases from 6 patients (3 women and 3 men) with slides available for review and material for FISH study were included in our study. The patients ranged in age from 31 to 75 years (average 60.3 years; Table 1). Five of the cases were FL including 1 case with concurrent DLBCL. The initial lesions involved lymph nodes, tonsil, parotid gland, and breast tissue. One patient (cases 2 and 3) initially presented with breast mass with diagnosis of DLBCL with signet-ring cell morphology. Four and half years later, the patient developed extensive lymphadenopathy and a thigh mass which was subsequently diagnosed as low-grade FL with signet-ring cell morphology.

3.2. Histopathologic features

Microscopic examination demonstrated all cases to contain numerous signet-ring cells with abundant clear or vacuolated cytoplasm (cases 1-5), or abundant eosinophilic, Russell body-like cytoplasm (cases 6 and 7). As shown in Fig. 1, the breast mass needle biopsy sample (case 2) showed diffuse proliferation of large, atypical polymorphic cells with irregular nuclear contours, dispersed or vesicular chromatin, one to several distinct nucleoli, and abundant clear cytoplasm. Mitotic figures and apoptotic bodies were easy to find. The biopsy (Fig. 2) of the left thigh mass (case 3) from the same patient 4 years later showed nodular proliferation of predominantly small atypical lymphoid cells with irregular nuclear contours, dispersed chromatin, inconspicuous nucleoli, and abundant clear cytoplasm in a background of delicate fibrosis and vascular proliferation. Few scattered large atypical lymphoid cells with vesicular chromatin and multiple nuclear membrane–associated nucleoli are present. In case 6, the right tonsil removed from a 53-year-old woman showed 3 areas with different morphology: 1 portion (not shown) with preserved follicular lymphoid architecture and 1 portion (Fig. 3A) with vaguely nodular proliferation of predominantly small atypical lymphocytes with condensed chromatin, slightly irregular nuclear contours, inconspicuous nucleoli, and moderately abundant clear cytoplasm. Scattered large atypical lymphoid cells and aggregates of plasma cells are noted. The third portion (Fig. 3B) contains sheets of large atypical lymphoid cells with vesicular chromatin and multiple inconspicuous nucleoli admixed with frequent large signet-ring-like cells containing round pink globules, resembling very large Russell bodies. Similar to case 6, case 7 (Fig. 4) showed nodular and diffuse proliferation of predominantly small atypical lymphoid cells with abundant eosinophilic cytoplasm pushing the nuclei to the periphery of cells.

3.3. Immunohistochemical findings

The results of IHC stains of the 7 cases are summarized in Table 2. The signet-ring cells in all 7 cases were positive for CD20 and also positive for PAX-5 in all the 6 cases stained for PAX-5, confirming that they were of B-cell lineage. There were preserved follicular dendritic cell (FDC) meshworks as demonstrated by positive CD21 and/or CD23 stains in the FL cases (cases 1, 3, 4, 5, and 7). On the contrary, there were no demonstrable FDC meshworks in cases with DLBCL (case 2) or in area with DLBCL (case 6). Interestingly, 2 of the 3 cases with low-grade FL were negative for CD10 but positive for BCL6 and/or MUM-1. The SRCL cells in cases 2 and 3 showed essentially identical immunophenotype, suggesting that they might be clonally related. In case 6, the signet-ring cells were positive for cytoplasmic κ and negative for λ (Fig. 3C and D). Furthermore, IHC (data not shown) showed that these signet-ring cells were also positive for CD138, IgD, and MUM-1, and negative for IgM, IgG, IgA, and cytokeratin (AE1/AE3). Although sharing similar morphology with case 6, the signet-ring cells in case 7 (data not shown) were negative for CD138, IgM, IgD, IgG, IgA, and κ and λ light chain. None of the neoplastic cells were positive for mantle cell lymphoma markers cyclin D1 and SOX11.

3.4. Fluorescence in situ hybridization findings

As our index case (case 6) showed complex changes including *BCL6* rearrangement seen in approximately 30% of cells analyzed in addition to increased copy numbers of *MYC* and *MALT1*, FISH studies for *BCL6*,

Table 1
Summary of clinicopathologic features and follow-up

Case	Age (y)	Sex	Site of biopsy	Stage	Diagnosis	Treatment	Follow-up
1	31	F	Parotid, LN	II	DLBCL, FL 3	R-CHOP ×6	Local recurrence at 20 mo
2	71	F	Breast	III	DLBCL	Chemotherapy ×8	Dead 4.5 y
3	75	F	Thigh mass	Unknown	FL, low-grade	Unknown	Dead, 4 mo later
4	60	M	Submandibular LN	Unknown	FL, 1-2	Resection + radiation	Free of disease
5	60	M	LN	Unknown	FL 3	Unknown	Not available
6	52	F	Tonsil	Unknown	DLBCL, MZL	Unknown	Not available
7	73	M	LN	IIIA	FL, 1-2	Observation	Stable

Abbreviations: LN, lymph node; MZL, marginal zone lymphoma; R-CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone, and rituximab.