Primary Rectal Mucosa-Associated Lymphoid Tissue Lymphoma: Report of a Case with Extensive FISH Study

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Abstract: Primary rectal mucosa-associated lymphoid tissue (MALT) lymphoma is a very rare entity with scarce cytogenetic/molecular genetic data. We presented a polypoid rectal MALT lymphoma in a 75 year-old male. The tumor comprised small atypical lymphocytes forming focal lymphoepithelial lesions and expressing CD20 but not CD5, CD23, CD43, or cyclin D1. They were negative for translocations involving *IGH*, *MALT1*, *BCL2*, *BCL6*, and *CCND1* genes, and the copy numbers of chromosomes 3, 12, and 18 were normal by fluorescent in situ hybridization. The patient was disease-free for 83 months after oral endoxan and prednisolone and concurrent local radiotherapy. With increasing awareness of this disease and accumulating reports on clinicopathological and molecular data, the pathogenesis, best treatment strategy and clinical course of such tumors will be uncovered.

Keywords: Fluorescence in situ hybridization, mucosa-associated lymphoid tissue lymphoma, non-Hodgkin lymphoma, rectum, Taiwan.

INTRODUCTION

Mucosa-associated lymphoid tissue (MALT) lymphoma is low-grade B-cell lymphomas with histological characteristics recapitulating those of MALT as exemplified by the Peyer's patches [1]. Excluding diffuse large B-cell lymphoma, most intestinal B-cell lymphomas are of MALT type with the majority arising in the small intestine and rarely in the colorectal region [2, 3]. Primary rectal MALT lymphomas account for less than 1% of MALT lymphomas with only 30 more cases been reported in the English literature [4, 5]. The majority was polypoid lesions with a favorable prognosis; however, cytogenetic/molecular genetic study has rarely been performed. So far the only chromosomal aberration detected in rectal MALT lymphoma is t(11;18), in only 11% of cases [6-8]. We presented a case of rectal MALT lymphoma with extensive immunohistochemical and fluorescent in situ hybridization (FISH) studies and long-term follow-up information.

REPORT OF A CASE

A 75 year-old male presented with bowel habit change for 7-8 months without body weight loss, fever, or night sweating. He had no history of inflammatory bowel disease or any major illness. Digital examination revealed a palpable tumor, 5 cm from anal verge. Physical examination was negative for lymphadenopathy. During operation, a wide-based, 3-cm polypoid tumor with a cauliflower appearance and easy touch bleeding was identified and polypectomy was

performed. A diagnosis of malignant lymphoma was made and staging procedures showed an IE disease. The LDH level was within normal ranges. The patient received oral cyclophosphamide and prednisolone and concurrent local radiotherapy for 4320 cGy in 15 fractions. He was free of lymphoma for 83 months.

Under low-power microscopic examination, the polypectomy specimen showed vaguely nodular lymphoid infiltrate markedly expanding the submucosa with extension into the muscularis propria (Fig. **1A**). The surface mucosa was intact without ulceration. Under high-power, occasional lymphoepithelial lesions were seen (Fig. **1B**). The medium-sized lymphocytes exhibited irregular nuclear contours and moderate amount of pale cytoplasm with inter-follicular infiltration around the residual reactive or colonized germinal centers (Fig. **1C**).

Immunohistochemical study was performed using the labeled streptavidin-biotin peroxidase method (LSAB kit, DakoCytomation, Carpinteria, CA, USA), and an antigen-retrieval technique was applied as needed for each specific antibody. The antibodies used were CD3, CD20 (clone L26), CD21 (1F8), bcl-2 (124), bcl-6 (PG-B6p), Ki-67 (MIB-1), IgD, IgM, MUM1 (MUM1p (DakoCytomation), CD43 (MT1) (BioGenex, San Ramon, CA, USA), CD5 (4C7), CD10 (56C6), CD23 (1B12), CD27 (137B4) (Novocastra, Newcastle upon Tyne, UK), cyclin D1 (SP4; LAB Vision Co., Fremont, CA, USA), p53 (DO-7) (Serotec Inc., Raleigh, NC, USA) and bcl-10 (in-house) [9]. Staining with anti-CD21 highlighted the follicular dendritic meshworks in the altered or colonized germinal centers, which were widely spaced due to inter-follicular infiltrate. The tumor cells expressed CD20, bcl-2, and IgM, but not CD3, CD5, CD10, CD23, CD27, CD43, bcl-6, IgD, cyclin D1, p53, or MUM1. Staining for bcl-10 showed weak to moderate cytoplasmic

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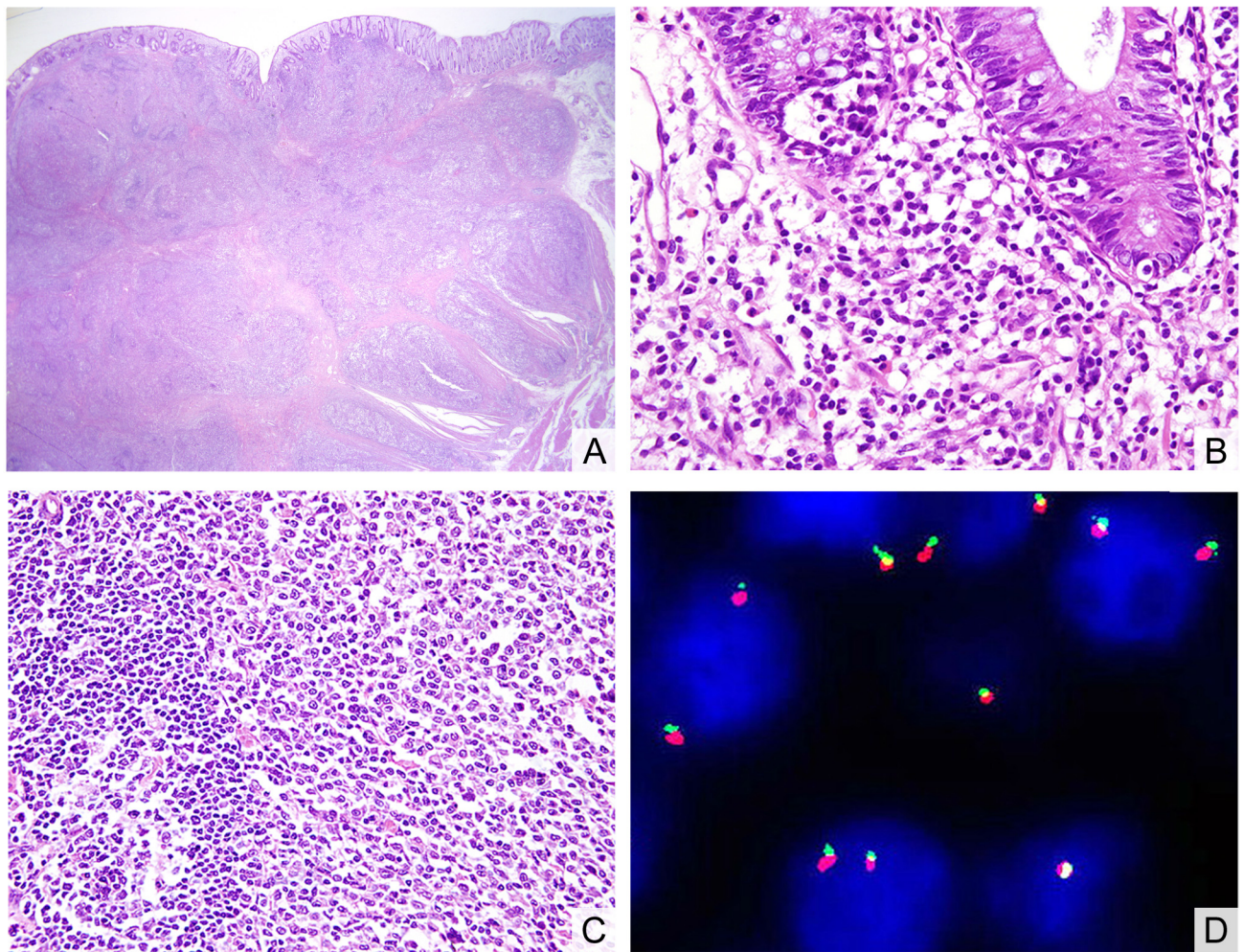
Fig. (1). Primary rectal mucosa-associated lymphoid tissue lymphoma. (**A**) Low-power view shows a polypoid lymphoid infiltrate with intact surface mucosa and extension into muscularis propria at the right lower corner. (**B**) Focal lymphoepithelial lesion. (**C**) High-power view of the atypical lymphocytes with slightly irregular nuclear contours on the right side with residual germinal center on the left. (**D**) Fluorescent in situ hybridization using *MALT1* dual color, break apart rearrangement probe. In tumor cells with *MALT1* rearrangment, there will be one fusion, one orange, and one green signal pattern indicative of a rearrangement of one copy of the *MALT1* gene region while two fusion signal pattern indicates negative results for translocation. In our case, the image shows two fusion signal pattern indicating negative results for *MALT1* rearrangement.

staining without nuclear signals. The proliferation fraction as determined by Ki-67 immunostaining was very low.

Locus-specific interphase FISH was performed on paraffin sections. Briefly, de-paraffinized sections were pre-treated by pressure-cooking for 3 minutes in EDTA (ethylenediaminetetraacetic acid) buffer (1 mM, pH 8.0) and subsequent incubation in pepsin solution for 25 minutes at 37°C to increase DNA accessibility. Sections were then dehydrated through ethanol and air-dried. The appropriate probe mix (1.0 μ l) was applied to the tissue section and covered with a round 10 mm cover slip. Both probe and target DNA were simultaneously denatured at 80°C for 25 minutes and incubated up to 2 days at 45°C. Post-hybridization washes were performed according to the "rapid-wash protocol" provided by Vysis, Downers Grove, IL. Sections were counterstained with 4,6-diamidino-2-phenylindole (DAPI) and mounted in Vectashield antifade solution (Vector Laboratories, Burlingame, CA, USA). Image acquisition and processing was per-

formed as previously described [10]. Six dual color, break apart rearrangement probes directed at *IGH*, *MALT1*, *BCL2*, *BCL6*, *CCND1* (Vysis/Abbott Laboratories Ltd, UK)*, and *FOXP1* (in-house) [11] genes and three centromeric enumeration probes (CEP) directed at chromosomes 3, 12 and 18 (Vysis/Abbott Laboratories Ltd.) were used. The tumor cells showed negative results for rearrangements involving *IGH*, *MALT1* (Fig. **1D**), *BCL2*, *BCL6*, and *CCND1* genes. Repeated study using probes for *FOXP1* rearrangement was unsuccessful. All three FISH assays using CEP 3, 12, and 18 probes showed two dots, indicating absence of trisomies 3, 12, or 18.

DISCUSSION

The differential diagnoses of this case include follicular lymphoma (FL) and mantle cell lymphoma (MCL). In addition to morphologic features, immunophenotype is helpful in excluding these two diagnoses by the negative expression of

CD10 and bcl-6 for FL and CD5, CD43, and cyclin D1 for MCL.

MALT lymphoma is associated with recurrent chromosomal translocations t(11;18)(q21;q21), t(14;18)(q32;q21), t(1;14)(p22;q32), and t(3;14)(p14.1;q32) involving *API2* (11q21), *IGH* (14q32), *MALT1* (18q21), *BCL10*(1p22), and *FOXP1*(3p14) genes [12, 13]. Rectal MALT lymphoma is very rare and usually appears as single case reports in the literature. In the earlier studies, two rectal MALT lymphomas with conventional cytogenetic studies were reported. Both tumors carried t(11;18), one as the sole chromosomal aberration and the other with an additional aberration of trisomy 3 [6, 7]. Using multiplex reverse transcription polymerase chain reaction (RT-PCR) method, Sakugawa *et al*. identified *API2-MLT1* fusion genes in 7/47 (15%) colorectal MALT lymphomas including 3/27 (11%) primary rectal tumors [8]. As compared to the *API2-MLT1*–negative cases, *API2-MLT1*–positive colorectal MALT lymphomas were larger in size, with male predominance and advanced clinical stages. In a most recent case report with literature review, Kabayashi *et al*. reported *API2-MLT1* fusion gene by RT-PCR in a young adult with primary rectal MALT lymphoma successfully treated with radiotherapy [5]. In that review of a total of 34 cases, the median age is 62 years with equal sex ratio and a predominance of polypoid tumors. Ulcerative lesion is far less common, while multiple mucosal lesions with simple reddish discoloration as reported by Lee *et al*. are extraordinary [14].

Using extensive FISH probes, we intended to look for other possible chromosomal translocations specific for MALT lymphoma in our case since so far no cytogenetic/molecular genetic studies other than t(11;18)/*API2-MLT1* have been reported. We found that our case was negative for rearrangements involving *IGH*, *MALT1*, *BCL2*, *BCL6*, and *CCND1* genes while the study for *FOXP1* gene rearrangement was unsuccessful. The failure to get satisfactory *FOXP1* signals may be due to sub-optimal quality of this in-house probe as compared to the commercial probes, although it had been successfully applied to paraffin sections previously [10, 15].

In summary, our patient had a solitary and polypoid rectal MALT lymphoma presenting with bowel habit change. He was cured with polypectomy, radiotherapy and low dose chemotherapy. No specific chromosomal translocation or trisomy commonly associated MALT lymphoma was identified by extensive FISH study. More reports of rectal MALT lymphomas with extensive searches for chromosomal aberrations and with long-term follow-up data such as reported here will help to uncover the pathogenesis, best treatment strategy and clinical course of such tumors.

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