

• CASE REPORT •

## Detection of *BCL2-IGH* rearrangement on paraffin-embedded tissue sections obtained from a small submucosal tumor of the rectum in a patient with recurrent follicular lymphoma

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### Abstract

A 59-year-old woman was admitted to our hospital because of recurrent follicular lymphoma (FL). Colonoscopic examination revealed a rectal submucosal tumor (SMT) without any erosions and ulcers. In this patient, it was difficult to distinguish non-Hodgkin's lymphoma (NHL) invasion from other disorders of the colon including carcinoid tumor merely based on endoscopic findings. Histopathologic and immunohistochemical studies on biopsy specimens showed an infiltration of atypical lymphocytes that were positive for CD20 and BCL2 but negative for UCHL-1. Fluorescence *in situ* hybridization on paraffin-embedded tissue sections (T-FISH) identified a translocation of *BCL2* with *IGH* gene. Based on these findings, the tumor was defined as an invasion of FL. T-FISH method is useful for the detection of a monoclonality of atypical lymphocytes in an SMT of the gastrointestinal tract, and particularly for the detection of chromosomal translocations specific to lymphoma subtypes.

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### INTRODUCTION

Primary gastrointestinal (GI) lymphomas are uncommon tumors, constituting less than 2% of all GI malignancies<sup>[1]</sup>. The stomach, however, is the most frequent site of extranodal NHL. Of all GI NHLs, the incidence of gastric lymphomas ranged from 51% to 86%<sup>[2,3]</sup>, while that of colonic lymphomas ranged from 1.6% to 16.2%<sup>[4,5]</sup>. Diagnosis of GI lymphomas may be complicated due to difficulties in pathological diagnosis and in staging with

endoscopic biopsies. In fact, it is occasionally difficult not only to distinguish NHL from other tumors but also to define subtypes of NHL on the basis of endoscopic and histological findings of biopsy specimens<sup>[6,7]</sup>. Although flowcytometric analysis is efficient for differential diagnosis of lymphomas, obtaining a diagnostic biopsy may sometimes be difficult because GI lymphomas spread submucosally and have normal surface qualities; therefore, a number of specimens are required for an accurate diagnosis.

On the other hand, chromosomal translocations are closely associated with distinctive subtypes of malignant lymphoma<sup>[8]</sup>. Both chromosomal banding and fluorescence *in situ* hybridization (FISH) are used for the detection of specific translocation. In addition, we have developed a procedure using FISH directly on paraffin embedded tissue sections (T-FISH), enabling us to perform cytogenetic analyses on both archival and small biopsy specimens<sup>[9,10]</sup>.

In the current study, using T-FISH we detected rearrangement of the *BCL2* with immunoglobulin heavy chain (*IGH*) gene on biopsy specimens obtained from a small rectal submucosal tumor (SMT), thereby differentiating follicular lymphoma (FL) from other neoplastic tumors of colon as well as other types of lymphoma.

### CASE REPORT

A 59-year-old woman was admitted to our hospital because of the swelling of multiple cervical lymph nodes. The patient had suffered from stage IV disease of FL since 1997. Although multiple chemotherapies including high dose regimen supported by autologous hematopoietic stem cell transplantation were administered, complete remission has not been achieved. At the current hospitalization, the patient showed elevated soluble interleukin-2 receptor (1 024 U/L), while no abnormal cells were detected in both the peripheral blood and bone marrow. Gallium-scintigraphy showed abnormal accumulation at cervical and supra-clavicular lesions. Gastroscopic examination revealed no remarkable abnormalities, whereas colonoscopic examination detected a small elevated lesion (7 mm×8 mm in size) on the rectum.

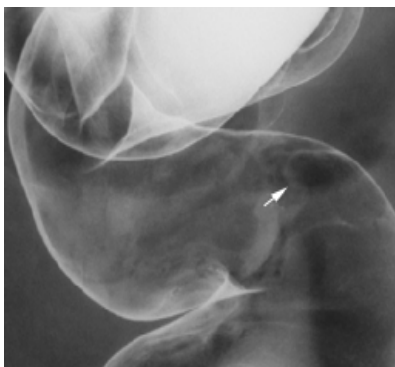
The surface of tumor was slightly reddish, but no erosions and ulcers were noted (Figure 1). No definitive deformity of colonic wall was detected with double-contrast barium enema (Figure 2). The tumor invasion was confined to the second layer of colonic wall based on the endoscopic ultrasonographic findings (Figure 3). Flowcytometric analyses were not able to be performed because of small specimens obtained from diagnostic biopsies. Histopathologic and immunohistochemical studies on biopsy specimens showed the aggregation of atypical medium-sized lymphocytes having irregularly enlarged nuclei that were positive for CD20, CD10 and BCL2, but negative for UCHL-1 and CD5 (Figures 4A-C).

Using DHAP regimen (cisplatin 70 mg/m<sup>2</sup>, Ara-C 1.4 g/m<sup>2</sup>×2, dexamethasone 40 mg×4), the patient achieved a partial response, followed by the administration of rituximab. The rectal lesion disappeared on d 120.

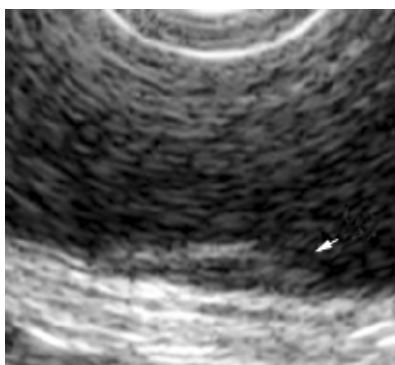
In order to confirm a diagnosis of lymphoma and define specific chromosomal abnormalities, we performed T-FISH analyses on the sample obtained from a rectal SMT according to the protocol already described elsewhere<sup>[9,10]</sup>. Briefly, sections from paraffin-embedded tissues were placed on slides, and then deparaffinized in xylene. Each slide was dehydrated in ethanol and treated with 0.2 mol/L HCl and 0.05 mg/mL proteinase K in TEN (0.05 mol/L tris-HCl, pH 7.8, 0.01 mol/L EDTA, and 0.01 mol/L NaCl buffer) for 10 min at 37 °C. FISH probes and samples were denatured simultaneously for 10 min at 90 °C, and were hybridized overnight at 42 °C. LSI IGH/BCL2, IGH/BCL1 and MALT1 probes (Vysis, CA, USA) were used for the detection of t (14; 18), t (11; 14), and 18q21 translocations, respectively. Images of signal were captured by a CCD camera. T-FISH detected fusion signals of *BCL2* and *IGH* genes on 75 of 100 nuclei (Figure 5), defining the invasion of FL with t (14; 18) (q32; q21). On the other hand, 95 of 100 nuclei showed negative results for both t (11; 14) and 18q21 translocations.



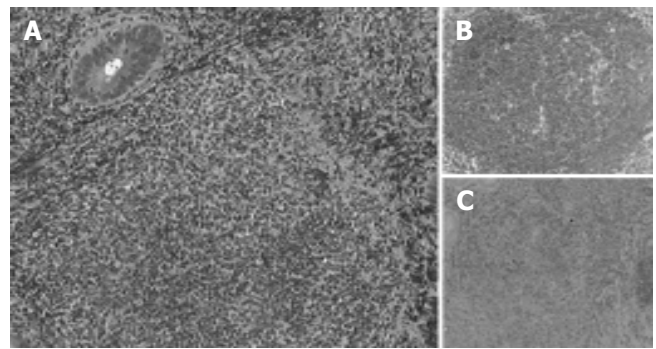
**Figure 1** Endoscopic examination revealed a small submucosal tumor on rectum.



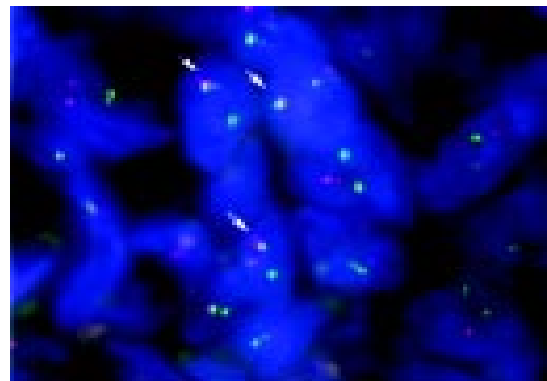
**Figure 2** Double-contrast barium enema revealed no definite deformity of the GI wall at the lesion.



**Figure 3** Endoscopic ultrasonography showed that the tumor was confined at the second layer of the colonic wall.



**Figure 4** Histopathological studies showed aggregated atypical lymphocytes (A). Immunohistochemical analysis revealed that these lymphocytes were positive for CD20 and BCL2 (B and C).



**Figure 5** T-FISH detected fusion signals of IGH and BCL2 genes in nuclei as indicated by arrows.

## DISCUSSION

We demonstrated rearrangement of *BCL2* with *IGH* gene on a small rectal SMT in a patient with recurrent lymphoma using T-FISH, thereby defining colonic involvement of FL. It is difficult to discriminate NHL from other disorders of the GI tract only based on endoscopic and histological findings<sup>[11]</sup>. Although endoscopic procedures are important not only in the detection of other disorders involving GI tract, but also in providing a means for pathologic diagnosis through biopsy, it should be noted, however, that obtaining a diagnostic biopsy may be difficult because GI lymphomas spread submucosally and have normal surface qualities. Since most GI lymphomas and other malignancies including adenocarcinoma and carcinoid tumor frequently display a polypoid growth pattern involving the mucosa, submucosa and muscularis<sup>[12]</sup>, it is sometimes difficult to make differential diagnosis based on the histological findings.

In the current patient, immunohistochemical studies defined a diagnosis of lymphoid lesion of a rectal SMT, although flowcytometric analysis was not successful because of small specimens obtained from diagnostic biopsy. There are many kinds of SMTs originating in the colon, for example, lipoma, malignant lymphoma, carcinoid tumor, *etc*<sup>[13]</sup>. Flowcytometric and immunocytochemical studies are useful for demonstration of a monoclonal population of lymphocytes of a lesion of interest, since most GI lymphomas are virtually always of the B-cell type. However, it should be noted that there are certain subtypes preferentially involving GI tract including mantle cell lymphoma, and mucosa-associated lymphoid tissue (MALT) lymphoma<sup>[14]</sup>. In this respect, cytogenetic study is necessary for the determination of these subtypes, since chromosomal translocations are closely associated with distinctive subtypes of NHL<sup>[14]</sup>. More than 90% of mantle cell lymphoma was related to the translocation of *IGH* with *BCL1* gene<sup>[15]</sup>. MALT lymphoma

was related to the translocation of *API2* with *MALT1* at frequencies of 18.8%<sup>[9]</sup>. Approximately 60% of FL showed the translocation of *BCL2* with *IGH* (Matsumoto *et al.* in press). Hence, T-FISH is a useful method not only to make definitive diagnosis of NHL but also to define its subtypes. In addition, a few biopsy samples are enough for T-FISH analysis when compared with flowcytometric analysis and polymerase chain reaction (PCR). Furthermore, the tissues embedded for a period of 15 years were available for T-FISH analysis<sup>[9,10]</sup>.

At present there is considerable controversy concerning the treatment of primary and secondary GI lymphomas. Rituximab has recently included in treatment option<sup>[16]</sup>. In our patient with recurrent FL, salvage therapy including high-dose Ara-C with rituximab was performed. Because *API2-MALT1*-positive lymphoma has demonstrated a more aggressive subgroup<sup>[17]</sup>, T-FISH will provide novel information for the selection of treatment for GI lymphomas.

In conclusion, our results indicate that T-FISH is a promising procedure for the routine detection of genetic alterations in GI lymphomas, it enables us to not only make definitive diagnosis of NHL but also to define its subtypes.

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