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Original Article

Demonstration of Chimeric DNA of *bcl*-2 and *Immunoglobulin Heavy Chain* in Follicular Lymphoma and Subsequent Hodgkin Lymphoma from the Same Patient

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We performed single cell polymerase chain reaction (PCR) amplification of the t (14 ; 18) translocation from paraffin embedded sections in a case of follicular lymphoma (FL) with subsequent development of Hodgkin lymphoma (HL). The lymphoma cells of FL were positive for CD20, CD10 and BCL-2, and negative for CD3, CD30 and CD15. Hodgkin and Reed-Sternberg (HRS) cells of HL were positive for CD20, CD30 and CD15, and negative for CD3 and CD10. EBER-1 RNA *in situ* hybridization failed to stain with both lymphomas. HRS cells manipulated and FL cells micro-shaved from individual neoplastic follicles were subjected to single-cell PCR. The t (14 ; 18) translocation, a chimeric DNA containing portions of the *bcl*-2 and the *immunoglobulin heavy chain* (*IgH*) genes, was amplified from four of 27 isolated HRS cells and two individual FL follicles. All t (14 ; 18) PCRs yielded products of the same size, and an identical nucleotide sequence including the t (14 ; 18) translocation was found in both FL and HRS samples. Thus, the data demonstrate the common clonal origin of FL cells and HRS cells in subsequent HL, and that both FL and HL were derived from germinal center B cells with the t (14 ; 18) translocation. 〔*J Clin Exp Hematopathol 47(1) : 9-13, 2007*〕

Keywords: Hodgkin lymphoma, follicular lymphoma, t (14 ; 18), composite lymphoma, single cell PCR

INTRODUCTION

Co-occurrence of non-Hodgkin's lymphoma (NHL) and Hodgkin lymphoma (HL) in the same patient is extremely rare, but this phenomenon occurs more frequently than would be expected by chance alone[1-5]. The most common presentation of such co-occurrence is nodular lymphocyte-predominant Hodgkin lymphoma (NLP-HL) with diffuse large B-cell lymphoma (DLBCL). In the case of classical Hodgkin lymphoma (CHL), the vast majority of co-occurring NHL is follicular lymphoma (FL)[1-5]. The elevated frequency of co-occurrence relative to chance alone suggests a clonal relationship between HL and DLBCL or FL when they present together. In previous studies, neither comparison of immunophenotypes between HL and FL nor Southern blotting analysis of the *immunoglobulin heavy chain* (*IgH*) gene gave clear evidence for or against clonality[5]. In contrast, single cell polymerase chain reaction (PCR) using micromanipulation of target cells has been used successfully to demonstrate a clonal relationship[6-8]. Abnormal cells of NLP-HL and the HRS cells of classical HL are of monoclonal B-cell origin[6-8]. Brauninger *et al*. have identified common germinal-center B cell precursors in two patients with both HL and NHL (FL and T-cell rich B-cell lymphoma, respectively)[9]. Marafioti *et al*. also reported that classical HL and FL were derived from a single clonal B cell[10]. Thus, a clonal relationship between co-occurring FL and HL has been previously demonstrated.

The translocation of t (14 ; 18) (q32 ; q21) associated with the overexpression of the Bcl-2 oncoprotein is found in approximately 80% of FLs[17], but the relationship of t (14 ; 18) to HL remains uncertain, as this cytogenetic abnormality has been reported in only a few cases of HL. Brauninger *et al*. did not assay for t (14 ; 18), and Marafioti *et al*. assayed for but did not detect the chimeric DNA of the *bcl*-2 and *IgH* genes resulting from t (14 ; 18)[10]. These findings were consistent with previous reports of the absence of t (14 ; 18) (q32 ; q21) in neoplastic HRS cells of classical HL[11]. So far, there has been no report of co-occurrence of FL and classical HL with t (14 ; 18).

In this study, we performed single cell amplification of the t (14 ; 18) translocation and the *IgH* gene in a case of FL with subsequent development of HL, using samples from a

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paraffin-embedded section. We demonstrate the same t (14 ; 18) translocation and *IgH* gene in both the HRS cells and FL cells, and conclude that the FL and HL had a common clonal origin in this patient.

MATERIALS AND METHODS

A resected tissue was fixed in 20% buffered formalin and embedded in paraffin for the histologic, immunohistochemical and molecular studies.

Molecular studies

1) Micro-manipulation from formalin-fixed, paraffin-embedded tissue and DNA extraction

Neoplastic follicles were shaved from a 10 μm section of FL, and the HRS cells in HL were micro-manipulated according to the methods previously described[12-14]. Target cells were lysed by incubation in an oil-covered drop of 25 μl distilled water + 1 μg/μl proteinase K (Roche Diagnostics, switzerland) as previously described[14].

2) PCR-amplification

A t (14 ; 18) translocation site involving the *bcl*-2 oncogene (chromosome 18) and *JH* region of the *IgH* gene (chromosome 14) was amplified, according to a previously described method[15]. Primers were as follows : 5'- TTA GAG AGT TGC TTT ACG TG -3', upstream of *bcl*-2 oncogene major break point cluster (*bcl*-2), and 5'- ACC TGA GGA GAC GGT GAC CAG GGT -3', a consensus J region primer (*JH*). PCR condition consisted of 1 cycle of 95℃ for 5 min, 61℃ for 4 min and 72℃ for 5 min and 40 cycles of 95℃ for 30 sec, 61℃ for 30 sec and 72℃ for 30 sec.

3) Nucleotide sequence analysis of the PCR products

Nucleotide sequence analysis of the PCR products was performed according to a previously described method[16].

RESULTS

Case's history, histology and immunohistochemistry

The patient was a 44-year-old Japanese male. Intra-abdominal mass and lymphadenopathy were identified in a hospital. Laparotomy and biopsy of the mass were undertaken and the resected tissue revealed that neoplastic follicles of various sizes were scattered throughout the lymphoid tissue. These neoplastic follicles consisted of a mixture of large and medium-sized lymphocytes, but not of tingible body macrophages, indicative of a follicular lymphoma, grade 2 (Fig. 1A). Karyotypic analysis showed 47, XX, t (14 ; 18) (q32 ; q21), +18 [3] /51, XX, idem, +2, +3, +21, +21 [2]. The lymphoma cells in neoplastic follicles were positive for CD20, CD10 and BCL-2, and negative for CD3, CD30 and CD15. The patient received combination chemotherapy until three years after the diagnosis. The lymphadenopathy be-

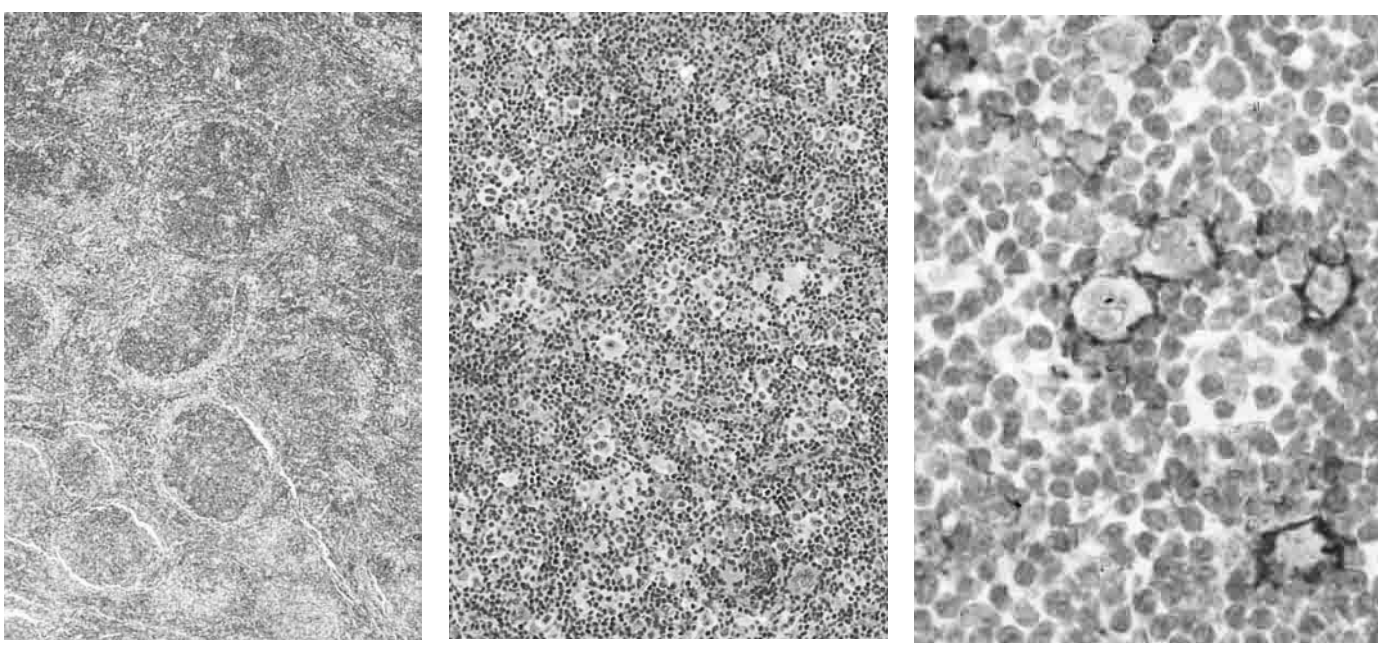

Fig. 1. Histology of follicular lymphoma and subsequent Hodgkin lymphoma. Intra-abdominal mass reveals various sized neoplastic follicles scattered throughout the lymphoid tissue, indicating follicular lymphoma. Immunohistochemistry of BCL-2 reacts with neoplastic follicles (*left*). After combined chemotherapy, a resected cervical lymph node showed mixed cellularity classical Hodgkin lymphoma (*center*). Mononuclear or multinucleated large-sized cells are occasionally seen in a background of numerous small non-neoplastic lymphocytes and positive for CD30 (*right*).

came smaller, but did not disappear. One year after the termination of chemotherapy, the patient experienced cervical lymphadenopathy and fever. The resected lymph node was almost effaced by a diffuse infiltration of lymphoid cells, which were comprised of numerous small non-neoplastic lymphocytes and scattered mononuclear or multinucleated large-sized cells. Typical Reed-Sternberg cells with a mirror-imaged nucleus and prominent nucleoli were occasionally seen. A diagnosis of mixed cellularity classical Hodgkin lymphoma (MC-CHL) was made (Fig. 1B, 1C). HRS cells showed immunoreactivity for CD20, CD30 and CD15. EBER-1 RNA *in situ* hybridization stained neither FL cells nor HRS cells.

Molecular studies

Two individual follicles shaved from FL and 27 isolated HRS cells from HL (Fig. 2) were processed for PCR-amplification of the t (14 ; 18) translocation. PCR products were found both in two samples from FL and four out of 27 samples from HL. All 6 PCR products showed a same sized band with about 170 bps sized length (Fig. 3). One sample each from FL and HRS were sequenced, revealing that the products spanned the t (14 ; 18) translocation and had a nearly identical sequence (1 difference over 171 bps) (Table 1).

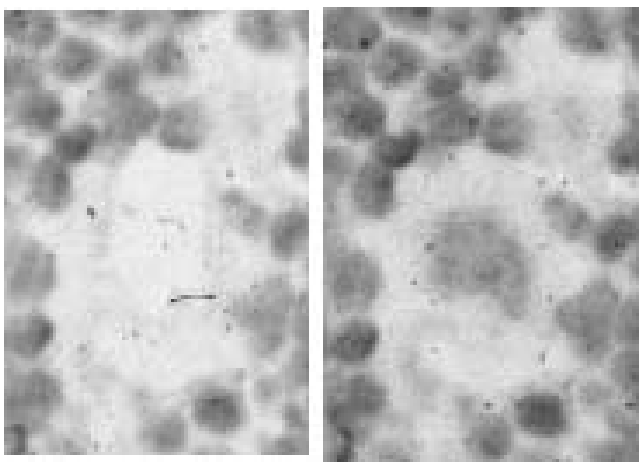

Fig. 2. Micromanipulation of a Reed-Sternberg cell. The target cell (*right*) is separated by a pulled pipette and lifted away. After micromanipulation of the target cell (*left*), the cell is no longer visible, but the remainder of the cells in the field are intact.

DISCUSSION

This study was conducted to detect the t (14 ; 18) translocation and the nucleotide sequence of the IgH gene in both FL cells and HRS cells from a patient of FL with subsequent HL.

Previous attempts to detect the *bcl-2/IgH* chimeric DNA by PCR have yielded ambiguous or conflicting results[18-20]. The chimeric gene was amplified from DNA extracted from HL-involved lymph nodes in a case of classical HL[19]. In contrast, Gravel *et al*. found that single cell PCR amplification of t (14 ; 18) from HRS cells yielded no PCR products in each of 10 examined cases[11]. The discrepancy between the relatively high rate of successful amplification of t (14 ; 18)

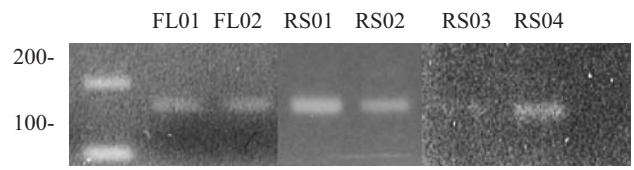

Fig. 3. PCR products of the t (14 ; 18) translocation site involving the *bcl*-2 oncogene (chromosome 18) and *JH* region of the *IgH* gene (chromosome 14). Two individual follicles shaved from FL and 27 isolated HRS cells from HL were processed for PCR amplification of the t (14 ; 18) translocation. PCR products were found in both two samples from FL (FL01, FL02) and four out of 27 samples from HL (RS01-RS04). All PCRs from the HRS cells yielded an identically sized band of about 170bps sized length.

Table 1. Nucleotide sequence of the PCR product of t (14 ; 18) translocation involving *bcl*-2 oncogene and immunoglobulin heavy chain gene.

FL (FL01)	ttagagagttgctttacgtggcctgtttcaacacagacccacccagagccctcctgccct
HL (RS02)	----------
	bcl-2 gene → 1 ← *JH*
FL (FL01)	ccttccgcgggggctttctcatggctgtccttcagggtcttcctgaaatgcagtgg gag
HL (RS02)	---------- -----
FL (FL01)	gactggttcgacccctggggccagggaaccctggtcaccgtctcctcaggt (171bps)
HL (RS02)	----------g---------- (171bps)

Nucleotide sequence of the PCR products of FL01 for follicular lymphoma (FL) and RS02 for Hodgkin lymphoma (HL) (Fig. 3) was determined. Chimeric DNA representing a fusion of the *bcl*-2 gene and *immunoglobulin heavy chain* gene (*JH*) was found. "-" indicates sequence identity.

using DNA extracted from lymph nodes and the low rate of amplification using DNA extracts from single cells might be explained by the presence of non-neoplastic cells with t (14 ; 18) translocation in HL-involved lymph nodes[11].

Lebrun *et al*. examined the translocation of t (14 ; 18) (q32 ; q21) by PCR amplification from lymph node DNA extract in a case with FL and subsequent HL, and both samples yielded a product of identical size and sequence[21]. This suggests a close relationship between FL and HL because same breakpoint, but it is not confirmed due to because of possibility that the presence of non-neoplastic cells with t (14 ; 18) translocation in HL-involved lymph nodes.

Recently, common clonal origin of both HL and FL in the same patient as demonstrated by single cell PCR-amplification by Brauninger *et al.*[9] and Marafioti *et al.*[10], respectively. However, Brauninger *et al.*[9] did not examine t (14 ; 18), and Marafioti *et al*.[10] did not detect PCR amplification of t (14 ; 18) in either the FL cells or the HRS cells. The case we describe here, therefore, is the first reported case with t (14 ; 18) in both FL and HL.

We tried to compare sequence of variable region of the IgH gene of both samples, too. An amplified *IgH* gene of the HRS cell showed the same nucleotide sequence except a few nucleotides as that of the preexisting FL (data not shown). Frequency of somatic mutation of both was approximately 10%. The similarity between these sequences indicates a common clonal origin of the HL and preexisting FL. The small difference between the sequences does not alter our interpretation, as it is known that a small number of mutations frequently occurs in a recurrent tumor[22].

The case reported by Brauninger *et al*. showed an identical sequence between the HRS cells and the FL cells[9]. On the other hand, the case reported by Marafioti *et al*.[10] was unusual. While sequences of the CDR3 region from both FL and HL were identical, those of variable region were different. Hence, the two clones were identical and derived from the germinal center B cells, but each of the two had its own somatic mutation pattern. Therefore, Marafioti *et al*. as well as Brauninger *et al*. demonstrate that clonally related cells may differentiate into HL and FL in the same patient[9,10]. With the case of FL and subsequent HL, the originating neoplastic clones with t (14 ; 18) may differentiate into HL and FL ; furthermore, it is possible that FL cells convert into HRS cells. Oncogenesis of HL following FL with or without t (14 ; 18) is still unknown and is likely to be same as that of classical *de novo* HL.

Subsequent Hodgkin lymphoma in patients with B-cell chronic lymphocytic leukemia may exhibit poorer clinical outcome relative to cases of *de novo* HL[23]. The outcome of composite lymphoma of follicular and Hodgkin lymphoma has not yet been reported. Unfortunately, we could not follow this patient because the patient stopped to go to the hospital.

In conclusion, we report here the first confirmed case of common clonal origin of HL and FL in the same patient. The implications for the biology of tumors of this kind are the lymphoma cells of low grade B-cell lymphoma could be converted to Hodgkin lymphoma.

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