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Case Study

Signet Ring Cell Lymphoma

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We present morphologic, immunohistochemical and molecular profiles of signet ring cell lymphoma (SRCL). The lymph node removed showed a diffuse infiltration of lymphoma cells consisting of large blastic cells (LBC) and signet ring cells (SRC). LBC had a large oval-shaped nucleus, occasionally with one or more nucleoli and scant cytoplasm, while SRC possessed eosinophilic cytoplasmic inclusions (Russell bodies) that displaced and distended the nucleus. LBC were CD20+, CD79a+, CD138− and CD10−. SRC were CD 20−, CD79a+, CD138+ and CD10−. Both LBC and SRC contained the same monoclonal cytoplasmic immunoglobulin (IgG, λ). Anti-CD 21 and anti-CD23 antibodies failed to stain the proliferative areas of the lymphoma cells. Somatic mutation and intraclonal microheterogeneity of the rearranged immunoglobulin heavy chain (IgH) gene variable region (VH gene) were examined. The VH gene exhibited 99% homology to the closest germline and no intraclonal microheterogeneity was found. These data revealed that the lymphoma cells possessed a class-switched IgH gene with little somatic mutation of the VH. This case is not a variant of follicular lymphoma derived from germinal center cells, but a variant of immunoblastic lymphoma derived from post germinal center cells.

Key words Signet ring cell lymphoma, B-cell, Immunoblastic, Somatic mutation, Immunoglobulin heavy chain gene

INTRODUCTION

Signet ring cell lymphoma (SRCL) is a rare variant of non-Hodgkin's lymphoma and is characterized by the proliferation of malignant lymphoid cells with cytoplasmic inclusions that displace the nucleus to the periphery of the cell. Kim *et al*. originally introduced the term "SRCL", because the lymphoma cells resemble mucin-producing carcinomas[1]. More than 50 cases of SRCL have been reported. SRCL is morphologically and immunohistochemically divided into three cytological subtypes : eosinophilic globule (Russell body), clear vacuole and hyaloplasmic deposit types[1−3]. The clear vacuole type SRCL is formed in either B- and T-cell lymphomas or null-cell anaplastic large cell lymphoma[4−6]. Russell body and hyaloplasmic deposit type SRCL have been reported to be derived from B-cell, because of their relation to immunoglobulin[3]. Morphologic and immunohistochemical studies have provided evidence that SRCL with a B-cell phenotype are derived from follicular center cells, and can be described as a rare variant of follicular lymphoma (FL)[7−9]. Signet ring cells, however, are found to exist in other B-cell malignancies such as immunoblastic lymphoma[2], Burkitt-like lymphoma[10], MALT-type lymphoma[11], Waldenström macroglobulinemia[12] and small lymphocytic lymphoma[13].

Analysis of the rearranged immunoglobulin heavy chain gene variable region (VH gene) is a powerful tool used to define the clonal origin of B cells and B-cell neoplasms[14−16]. Pre-germinal center (GC) B cells and their neoplasms exhibited the germline configuration of the rearranged VH gene, whereas GC & post-GC B cells and their neoplasms have a somatically hypermutated VH

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gene. Intraclonal microheterogeneity is frequently identified in FL.

We examined somatic mutation of the rearranged VH gene of B-cell SRCL to define its cellular origin. The rearranged VH gene of this case exhibited little somatic mutation, as well as no intraclonal microheterogeneity, suggesting that this SRCL was a variant of immunoblastic lymphoma, but not of FL.

CASE REPORT

A 60-year-old Japanese woman was admitted to Fukushima Medical University Hospital complaining of cervical lymphadenopathy. A diagnosis of signet ring cell lymphoma was made by a biopsy of the cervical lymph node. The patient received combination chemotherapy (cyclophosphamide, vincristine, adriamycin, and prednisolone, CHOP) and progressed to complete remission. The patient lived further 11 years with a few recurrences in the stomach, peripheral blood and bone marrow, and eventually died of pneumonia.

MATERIALS AND METHODS

Tissue samples taken from the lymph node for light microscopy were fixed in 10% formaldehyde solution and embedded in paraffin. Immunohistochemical studies were performed on the paraffin-embedded tissues and frozen tissues using the strept-avidin biotin complex technique[17]. In this study, the primary reagents used included monoclonal and polyclonal antibodies against CD3, CD4, CD5, CD10, CD20, CD21, CD23, CD30, CD43, CD56, CD79a, CD138, Bcl-2, Bcl-6, P53, TIA-1, MIB-1, IgG, IgA, IgM, IgD, κ and λ. DNA was extracted from the frozen tissue. The methods for PCR-amplification, nucleotide sequence and subcloning assay of the VH gene have been described previously[18].

PATHOLOGIC FINDINGS

Morphology

The lymph node showed effacement of normal lymph node architecture by a diffuse infiltration of atypical lymphoid cells. Atypical cells consisted of large blastic (LBC) and signet ring cells (SRC). The number of SRC was greater than that of LBC. LBC had a large nucleus with dispersed chromatin and occasionally one or more eosinophilic nucleoli. A small number of mitotic cells were seen. SRC contained intracytoplasmic eosinophilic inclusions displacing nuclei to the periphery of the cytoplasm and resembled a signet ring morphology (Fig. 1). The inclusions were diastase-resistant PAS-positive. Under electron-microscope inspection SRC had abundant dilated cisternae of rough endoplasmic reticulum (ER) and most of them contained regularly arranged crystalline substances (Fig. 2). We found that a small number of the transitional cells between LBC and SRC possessed rough ER and a slightly indented nucleus.

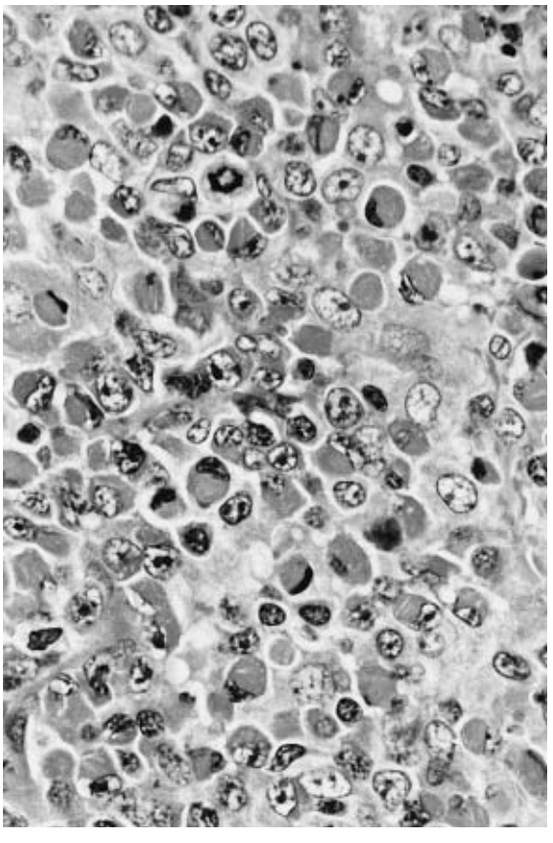

Fig. 1. A histological view of signet ring cell lymphoma
Signet ring cell lymphoma is comprised of large blastic cells (LBC) having a large nucleus with dispersed chromatin and occasionally one or more eosinophilic nucleoli and signet ring cells (SRC) containing intracytoplasmic eosinophilic inclusions displacing nuclei to the periphery of the cytoplasm.

Immunohistochemistry

Immunohistochemical studies revealed monoclonal cytoplasmic immunoglobulin (CIg) of IgG, λ in both LBC and SRC. LBC were of CD20+CD79a+ B-cell and co-expressed CD30. SRC had the immunophenotype of plasma cells: CD79+ CD20− CD138+. The lymphoma cells did not express CD10. Because CD21 and CD23 failed to stain in the same place where lymphoma cells are located, no network pattern of follicular dendritic cells (FDC) was found. (Table 1)

Molecular, VH gene-analysis

The direct sequence of the PCR-amplified VH gene showed 99% homology to the germline of DP21, VH1 family. The incidences of somatic mutations were 1/15 in CDR1, 0/42 in FW2, 0/51 in CDR2 and 1/96 in FW3 (expressed as the number of mutations to the number of nucleotides). These two single nucleotide substitutions were a replacement mutation. A subcloning assay of the PCR-amplified sample was performed for analysis of intraclonal microheterogeneity. There were no nucleotide substitutions in the nine clones examined. (Table 2)

Table 1 Immunohistochemical stuidies of signet ring cell lymphoma

	Large blastic cells	Signet ring cells	Source
CD3	−	−	DC
CD4	−	−	NC
CD5	−	−	NC
CD8	−	−	DC
CD10	−	−	NC
CD20	+	−(*)	BD
CD21	−	−	DC
CD23	−	−	SE
CD30	+/−	−	DC
CD43	−	−	BS
CD56	−	−	ZY
CD79a	+	+	DC
CD138	−	+	SE
Bcl-2	−	−	DC
Bcl-6	−	−	SC
P53	−	−	DC
TIA-1	−	−	IT
MIB-1	+/−	+/−	IT
CIg	IgG, λ	IgG, λ	DC

+, positive cells > 50% of cells ; +/−, 20% < positive cells < 50% ; −, negative ; −(*), positive cells were found less than 10% of the cells.
CIg : Cytoplasmic immunoglobulin
DC : DakoCytation, Denmark
NC : Novocastra Laboratories, UK
SE : Serotec, Sapporo, Japan
BD : Becton Dickinson Pharmingen, USA
BS : QED Bioscience inc., USA
ZY : Zymed Laboratories Inc., USA
SC : Santa Cruz, USA
IT : Immunotech, USA

DISCUSSION

SRCL is well known to be a B-cell malignancy, except in rare cases of T-cell and null-cell SRCL[1−6]. Most SRCL have been described as a variant of FL[1−3,7−9], because SRC are occasionally found in normal GC and some cases of SRCL partially exhibited nodular architecture. Uccini *et al*. reported an immunohistochemical study of SRCL that revealed neoplastic nodules with a meshwork of DRC-1+ FDC and numerous SRC in the internodular area[9]. On the other hand, immunoblastic lymphoma[2], Burkitt-like lymphoma[10], MALT-type lymphoma[11], Waldenström macroglobulinemia[12] and small lymphocytic lymphoma[13] could have a plasmacytic differentiation with signet ring cell morphology in their neoplastic cells. The cellular origin of B-cell SRCL may not be simple. There has been no report that defines their origin using molecular techniques.

In our case of B-cell SRCL, Russell body type, the tumor consisted of a diffuse infiltrate of large blastic cells having a large nucleus with dispersed chromatin and occasionally one or more eosinophilic nucleoli, plasmacytic large cells and signet ring cells without a nodular pattern. The architectural and cytological features of this case differed from those of FL and were similar to those of immunoblastic lymphoma. Thus, the present case was considered to be immunoblastic lymphoma with a plasmacytic differentiation appearing as signet ring cell morphology. The immunohistochemistry revealed that lymphoma cells were negative for CD10 & Bcl-2 and FDC were not found within the proliferation areas of the lymphoma cells.

The sequence of the rearranged VH gene contained only two mutations among 204 nucleotides with respect to the closest germline, exhibiting 99% homology to the germline of

Table 2 Somatic mutation and intraclonal microheterogeneity of the immunoglobulin heavy chain gene variable region of signet ring cell lymphoma

germline/DP21	agc	tat	gct	atg	aat	(CDR1)												
consensus	-a-	---	---	---	---													
clone 1	-a-	---	---	---	---													
clone 2	-a-	---	---	---	---													
clone 3	-a-	---	---	---	---													
clone 4	-a-	---	---	---	---													
clone 5	-a-	---	---	---	---													
clone 6	-a-	---	---	---	---													
clone 7	-a-	---	---	---	---													
clone 8	-a-	---	---	---	---													
clone 9	-a-	---	---	---	---													
germline/DP21	tgg	gtg	cga	cag	gcc	cct	gga	caa	ggg	ctt	gag	tgg	atg	gga	(FW2)			
consensus	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
clone 1	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
clone 2	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
clone 3	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
clone 4	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
clone 5	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
clone 6	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
clone 7	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
clone 8	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
clone 9	---	---	---	---	---	---	---	---	---	---	---	---	---	---				
germline/DP21	tgg	atc	aac	acc	aac	act	ggg	aac	cca	acg	tat	gcc	cag	ggc	ttc	aca	gga	(CDR2)
consensus	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
clone 1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
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clone 8	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
clone 9	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
germline/DP21	cgg	ttt	gtc	ttc	tcc	ttg	gac	acc	tct	gtc	agc	acg	gca	tat	ctg	cag	(FW3)	
consensus	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
clone 1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
clone 2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
clone 3	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
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clone 8	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
clone 9	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
germline/DP21	atc	tgc	agc	cta	aag	gct	gag	gac	act	gcc	gtg	tat	tac	tgt	gcg	aga	(FW3)	
consensus	---	a--	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
clone 1	---	a--	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
clone 2	---	a--	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
clone 3	---	a--	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
clone 4	---	a--	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
clone 5	---	a--	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
clone 6	---	a--	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
clone 7	---	a--	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
clone 8	---	a--	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
clone 9	---	a--	---	---	---	---	---	---	---	---	---	---	---	---	---	---		

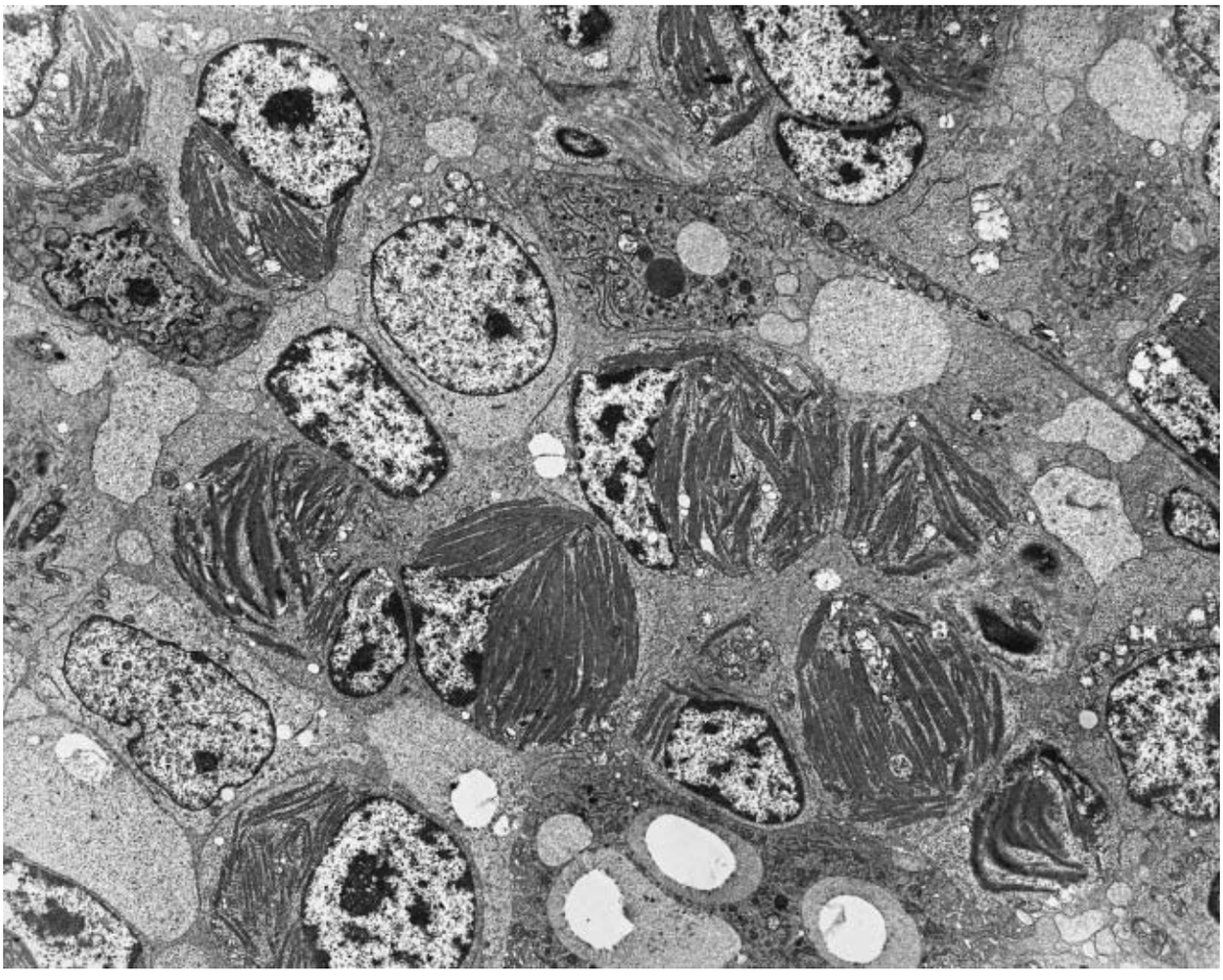

Fig. 2. An electronmicroscopic view of signet ring cell lymphoma.
Large blastic cells (LBC) and signet ring cells are intermingled. LBC contain a prominent nucleus and loose chromatin and SRC contain abundant cisternae of rough endoplasmic reticulum that are distended by regularly-arranged crystalline substance. Cells in the transitional stage between SRC and LBC were also seen.

DP21. The frequency of 99% homology to the closest germline is found in most lymphoblastic lymphoma (LL) and mantle cell lymphoma (MCL), more than half of the cases of B-CLL, and occasionally in diffuse large B-cell lymphoma (DLBCL), plasmacytic lymphoma and plasma cell myeloma, but rarely in FL and MALT-type lymphoma[15,16]. Because IgG was expressed in this case, an isotype switch took place, suggesting a GC or post-GC cell origin. Pre-GC cell neoplasms of LL and MCL are not a likely origin for this case. Moreover, no substitution in the cloning assay was identified. FL invariably has intraclonal microheterogeneity[16].

Recently, Lossos *et al*. reported that two distinct tumor subtypes of DLBCL, GC B cells-like DLBCL and activated B cells-like DLBCL were identified by gene expression profiling using DNA microarray markers[19]. Whereas germinal center B cells-like DLBCL exhibit intraclonal microheterogeneity, activated B cells-like DLBCL did not[19]. Thus, we failed to demonstrate any evidence for a GC B-cell origin in this case. The lymphoma cells had, apparently exclusive, findings of little somatic mutation of the VH gene and class switch. Although the lymphoma cells may undergo little somatic mutation in the GC, this may be explained by extra-follicular differentiation of pre-GC B-cells to become plasma cells. It is clear, at least, that the lymphoma cells were not derived from GC B-cells, but had characteristics of post-GC B-cells.

Our patient's recurrent stomach tumor showed a diffuse proliferation of the lymphoma

cells without a follicular pattern. LBC and plasmacytic cells were intermingled, but no SRC were found. Immunohistochemical and molecular data demonstrated the recurrence of SRCL (data not shown).

In conclusion, we report a case of B-cell SRCL, Russell body type, that revealed a proliferation of immunoblasts and plasmacytic cells showing SRC features. The immunologic and molecular data revealed that this SRCL was a variant of immunoblastic lymphoma derived from post-GC cells. There was no evidence that this case originated from FL or germinal center cells.

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