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Histological Progression of Follicular Lymphoma Associated with p53 Mutation and Rearrangement of the C-MYC Gene

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ABSTRACT

Follicular lymphoma is a low grade malignant lymphoma. However, some follicular lymphomas undergo histological transformation into higher grade malignant lymphomas. We recently encountered a diffuse large cell lymphoma which seemed to have progressed from a follicular lymphoma and which finally transformed into a small non-cleaved lymphoma. Each stage of the histological transformation was accompanied by increasing clinical grades of malignancy. It was suspected that in our patient a follicular lymphoma initially developed due to rearrangement of the BCL2 gene, and then underwent histological transformation into a diffuse large cell lymphoma, which was associated with p53 mutation. Subsequent rearrangement of C-MYC promoted the histological transformation of this diffuse large cell lymphoma into a small non-cleaved lymphoma. Our findings indicate that p53 mutation and rearrangement of C-MYC are involved in the histological transformation of follicular lymphomas into more advanced lymphomas.

Key words: *Follicular Lymphoma, Transformation, BCL2, p53, C-MYC*

Characteristic chromosomal abnormalities have been identified in the various forms of malignant lymphomas. Identification of the genes activated by chromosomal abnormalities and clarification of their action mechanisms have seen rapid progress in recent years. t (14;18) (q32;q21) is a chromosomal abnormality observed in follicular lymphomas (FL), and the anti-apoptosis BCL2 gene becomes activated as a result of this chromosomal translocation[10,25,26]. Most FL are of low grade malignancy, but some undergo histological transformation into diffuse lymphomas, which are more treatment-resistant than FL, following obliteration of their follicular pattern. The molecular mechanism of this histological transformation has not yet been clarified. We describe a patient in whom p53 mutation and rearrangement of C-MYC may have been responsible for the histological transformation of the FL into a diffuse large cell lymphoma (DL) and finally into a small non-cleaved lymphoma (SNC).

CASE REPORT

A 65-year-old male first noticed swelling in his right submandibular region in 1989. In October 1990, the patient was admitted to our hospital due to a fever elevation (37°C) which was unresponsive to antibiotics. On admission, multiple lymph nodes with a maximal dimension of 4cm were palpated in the cervical and submandibular regions bilaterally. The liver was palpated 5cm under the right costal margin and the spleen was palpated 4cm over the left midclavicular line. Hematological examination showed a WBC of 8,100/μl with no atypical cells, Hb of 8.0g/dl, and a platelet count of 3.4×10^4/μl. Biochemical tests revealed increases in GOT to 105U/liter, GPT to 78U/liter, LDH to 1,106U/liter, Al-P to 231U/liter, LAP to 78U/liter, CRP to 28.8mg/dl, beta-2-microglobulin to 8,490ng/ml, and ferritin to 1,034ng/ml. The serum IgM level was increased to 707mg/dl and M-protein of the IgM, κ chain was detected by immunoelectrophoresis. A small part of the

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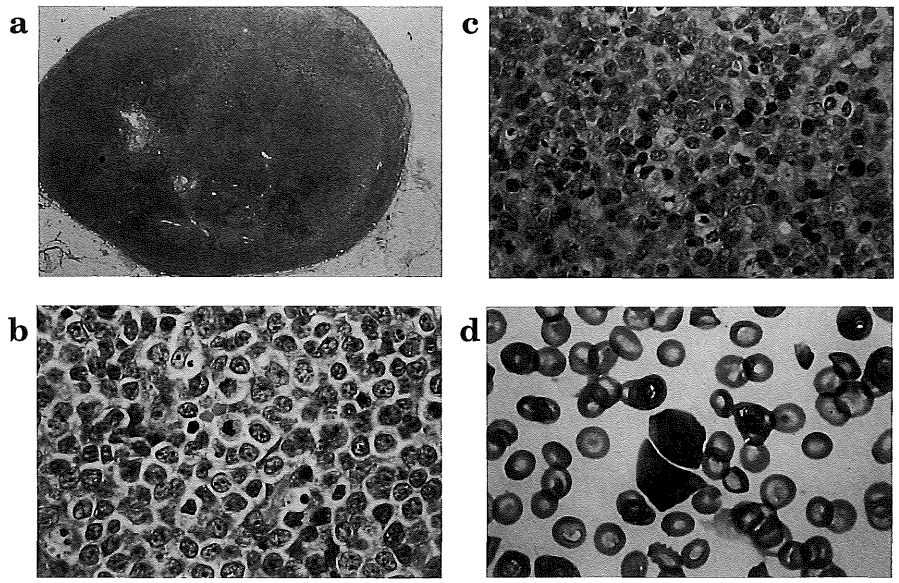

Fig. 1. (a) Cross-sectional surface of the submandibular lymph node. (b) Histopathological section of the submandibular lymph node. Malignant lymphoma, diffuse, large cell, non-cleaved (Working Formulation, intermediate grade) (c) Histopathological section of the tumor arising in the post nasal cavity. Malignant lymphoma, small non-cleaved (Working Formulation, high grade) (d) Small non-cleaved lymphoma cells observed in the peripheral blood at the terminal stage.

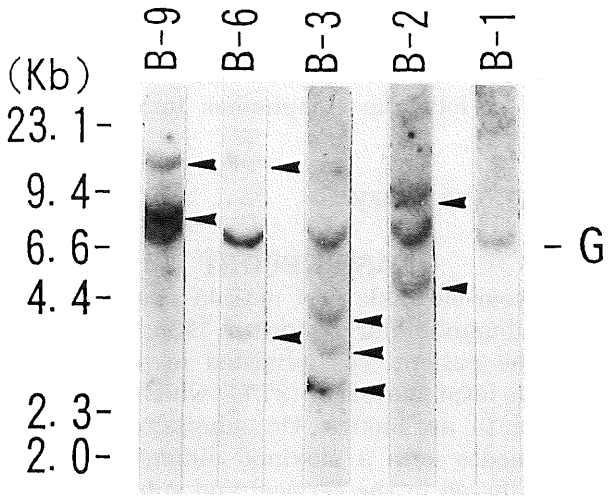

Fig. 2. Southern blot analysis of the heavy chains of immunoglobulin. DNA was digested with the restriction enzymes *Bam*H1 and *Hin*dIII and hybridized to a probe in the JH region. A rearrangement band (<) was detected in our patient (B-6) using DNA extracted from the submandibular lymph node (diffuse lymphoma, large cell, non-cleaved). B-1: follicular lymphoma, small cleaved, B-2, B-3: diffuse lymphoma, large cell, B-9: immunoblastic lymphoma.

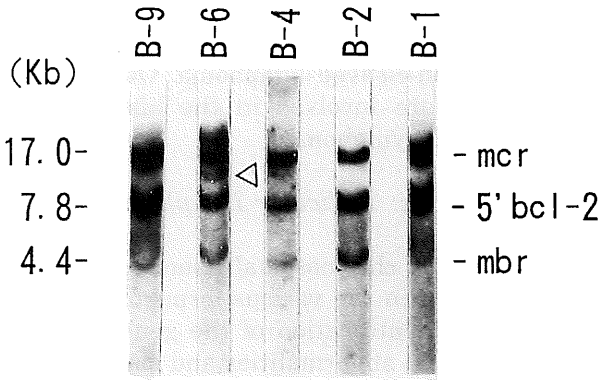

Fig. 3. Southern blot analysis of the BCL2 gene
DNA was digested with the restriction enzyme *Hin*dIII and hybridized to a mixed probe of mbr, mcr and 5'bcl-2. A rearrangement band (<) was detected in our patient (B-6) using DNA extracted from the submandibular lymph node (diffuse lymphoma, large cell, non-cleaved). B-4: immunoblastic lymphoma, plasmacytoid.

biopsy specimen of the left submandibular lymph node showed a follicular pattern. However, the greater portion of the lymph node showed a diffuse proliferation of large lymphoma cells with high mitotic rates (Fig. 1a, b). The large lymphoma cells were CD20 (+), CD43 (–), CD45RO (UCHL-1) (–), CD68 (KP-1) (–), CD3 (–), CD5 (–), CD4 (–), CD8 (–) and CD19 (–). Rearrangement of the immunoglobulin heavy chain gene (Fig. 2) and the BCL2 gene (Fig. 3) were observed. No rearrangement was observed in the κ and λ chains of immunoglobulin, the β and γ chains of T cell receptors, or in the C-MYC gene (Fig. 4).

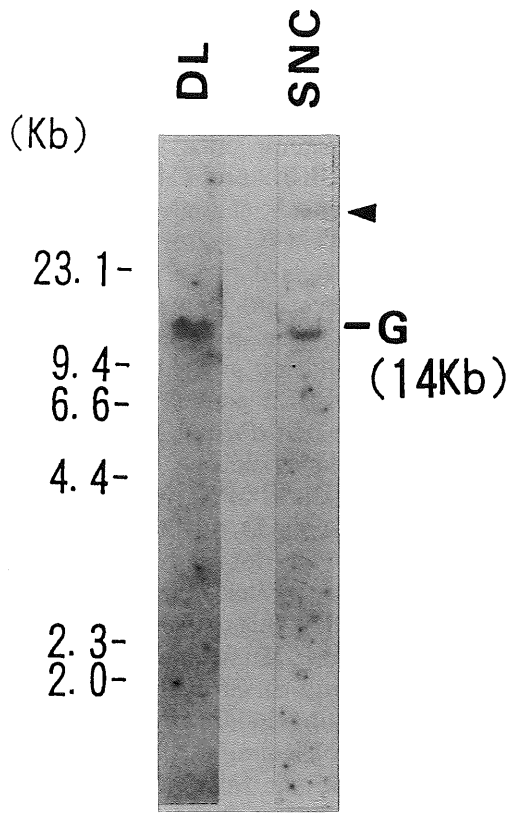

Fig. 4. Southern blot analysis of the C-MYC gene
DNA was digested with the restriction enzyme *ECO*RI and hybridized to the exonII portion of the C-MYC gene. DL: DNA extracted from the submandibular lymph node (diffuse lymphoma, large cell, non-cleaved). SNC: DNA extracted from lymphoma cells collected from the peripheral blood after transformation into a small non-cleaved lymphoma in the terminal stage.

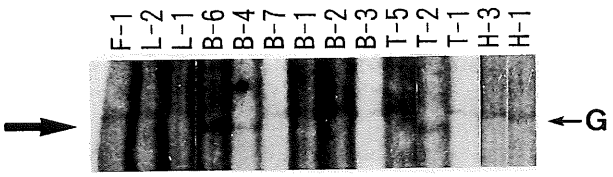

Fig. 5. Analysis of p53 mutations by single strand conformation polymorphism (SSCP)[22]
After synthesizing cDNA from RNA, PCR was performed with the primers 337–392 and 777–758. After denaturing into single strands, the samples were loaded on sequencing gel for analysis. Mutation of the p53 gene (→) was confirmed in our patient using RNA extracted from the submandibular lymph node (diffuse lymphoma, large cell, non-cleaved). R: normal control, HD: Hodgkin's disease, T: T cell type malignant lymphoma, B: B cell type malignant lymphoma, L: acute lymphocytic leukemia, F: blastic crisis of myelofibrosis.

Analysis of the p53 gene by single strand conformation polymorphism (SSCP) showed the presence of mutations between bases 373 and 758 (Fig. 5). A diagnosis of a malignant lymphoma, diffuse, large cell, non-cleaved cell (Working Formulation, intermediate grade) was made. Also it was suspected that a FL had undergone histological transformation into a DL, since a vague follicular pattern existed.

The superficial lymph nodes disappeared after chemotherapy with cyclophosphamide, doxorubicin hydrochloride, vincristine and prednisolone. However, splenomegaly persisted in the patient, and a splenectomy was performed on February 1991. Histological examination showed that large lymphoma cells had infiltrated the marginal zones of the spleen. Despite treatment with mitoxantrone, cyclophosphamide, cisplatin and prednisolone, the patient developed a tumor in the postnasal cavity. Histological examination of this tumor revealed immature cells (CD20 (+), CD43 (–), CD45R0 (–)), a high mitotic rate, and many tingible body macrophages. A diagnosis of a malignant lymphoma, small non-cleaved (Working Formulation, high grade) was made, indicating that the DL had transformed into a SNC (Fig. 1c). We attempted various therapies, but the patient gained only temporary relief. The WBC of the peripheral blood had almost all been replaced by small non-cleaved lymphoma cells during the terminal stage of our patient (Fig. 1d). The patient died in August 1991. The small non-cleaved lymphoma cells in the peripheral blood immediately before the death of the patient showed rearrangement of the C-MYC gene, which was not observed at the DL stage (Fig. 4).

DISCUSSION

From the clinical course and the histological findings, it was suspected that the FL in our patient underwent histological transformation initially into a DL, and finally into a SNC. Rearrangement of the BCL2 gene and IgH, and mutation of the p53 gene followed by rearrangement of the C-MYC gene were observed in this process.

The BCL2 gene was identified during analysis of the (14;18) (q32;q21) translocation which is characteristic of FL[25]. Rearrangement of the BCL2 gene is detected in 89% of FL in the United States and 46% of those in Japan. The genetic products of the BCL2 gene are localized in the mitochondrial and nuclear membranes of cells, and the function of this gene is to inhibit apoptosis[10,26]. The antiapoptotic action of the BCL2 gene is mediated by the antioxidant of the BCL2 protein[11] and by binding with the Bax protein which mediates cellular death[17]. The BCL2 gene is not expressed in the dark and basal light zones, but expressed in a small portion of the apical light zone of the lymphoid follicles[12]. That is,

the BCL2 gene is only expressed in cells which have undergone positive selection by the antigen presented by the follicular dendritic cells. The cells in which the BCL2 gene is expressed are spared from apoptosis, and subsequently differentiate into immunoblasts and memory B cells[13]. The overexpression of the BCL2 gene product in transgenic mice results in the inhibition of apoptosis in the pro-B cells[10,26]. This leads to an abnormal persistence of the mature B cell population in the lymph nodes and bone marrow[15,21]. In FL, the BCL2 gene is mainly cleaved at the 3'portion of the non-translated region (mbr), but is also cleaved in the extragenic region downstream to the 3' end (mcr) or in the 5' promoter region (5'bcl-2)[3,18,27]. The BCL2 gene activated by binding with the IgH gene expresses its product extraordinarily on a constant basis with resulting FL[16].

Some FL transform into DL following obliteration of their follicular pattern. Rearrangement of the BCL2 gene has been observed in 30% of American patients with DL[27] and in 9% of Japanese patients with DL[14]. The histological transformation into DL is usually accompanied by complex chromosomal abnormalities other than t (14;18)[29]. From these observations, a DL with rearrangement of the BCL2 gene is diagnosed as a lymphoma transformed from FL. In our patient, mutation of the p53 gene was detected during this histological transformation from FL to DL. The p53 is a transcription factor which binds to DNA. In response to DNA damage, the p53 levels rise and arrest the cell cycle at the G1 stage, thereby allowing time for DNA repair to occur. Thus, the normal p53 gene acts as a suppressor gene in malignant cells. However, genetic abnormalities accumulate in cancer cells with abnormal p53 genes. Coco et al[4] and Sander et al[19] detected mutation of the p53 genes in patients with DL which had transformed from FL. However mutation of the p53 gene is not found in "de novo" DL[9]. It has also been reported that mutation of the p53 gene enhances the expression of the multidrug resistant gene (MDR-1)[2]. The expression of this gene may account for the poor response to treatment in our patient.

The rearrangement of the C-MYC gene was thought to be responsible for the histological transformation of FL into malignant lymphomas of a higher grade[6,24]. However, it was later reported that only 8% of patients whose FL had undergone histological transformation showed rearrangement of the C-MYC gene[28]. In our patient, rearrangement of the C-MYC gene, which was not observed during the histological transformation from FL to DL, was observed after transformation to SNC in the terminal stage. An experimental study has shown that rearrangement of the C-MYC gene was present in half of the malignant lymphomas developed in BCL2 transgenic mice[16]. Moreover extremely undifferentiated lymphomas developed in doubly BCL2/C-MYC transgenic mice[21]. These observations suggest that BCL2 and C-MYC genes interact in the pathogenesis of high grade malignant lymphomas. The C-MYC gene has mainly been investigated with regard to its action in promoting cellular proliferation. However, recently, the role of the C-MYC gene in apoptosis has become clear[7,20]. The inhibition of C-MYC gene-induced apoptosis by the BCL2 gene[1,8] may have been responsible for the transformation into malignant lymphomas of higher grades.

Translocation of the C-MYC gene from chromosome 8 to chromosome 14 results in activation in de novo SNC[5,23]. The latent membrane protein of the EB virus, which has been causally associated with SNC, Burkitt type, activates cell-derived BCL2 genes. Mutations of the p53 gene are commonly found in SNC, Burkitt type[9]. In our patient rearrangement of the BCL2 gene, mutation of the p53 gene, and rearrangement of the C-MYC gene in the terminal stage all interacted in the transformational process of FL to a high grade malignant lymphoma (SNC). It needs further analysis of the effect of abnormal C-MYC and p53 gene products on the genes of the cell membrane and cytoskeleton proteins to clarify the mechanism of morphological change in the transformation.

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