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# Mutations of *p53*, c-*kit*, K-*ras*, and β-Catenin Gene in Non-Hodgkin's Lymphoma of Adrenal Gland

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**Malignant lymphoma of the adrenal gland is a rare disease, usually with diffuse large cell morphology and B-cell immunophenotype, and often associated with Epstein-Barr virus infection. In this study, mutations of *p53*, c-*kit*, K-*ras*, and β-catenin gene were analyzed in 17 cases (13 males and four females with ages ranging from 25 to 84 years) of such lymphomas by polymerase chain reaction-single strand conformation polymorphism followed by direct sequencing. Selected exons in each gene, representing hot spots, were analyzed. All 44 mutations detected were single-nucleotide substitutions and 33 were missense mutations. Nineteen mutations were detected in exon 5 and/or 7 of the *p53* gene in nine of 17 cases (52.9%) and 21 in exon 11 and/or 17 of the c-*kit* gene in 10 of 14 cases (71.4%). Bilateral adrenal lesions in one case who had not received any adjuvant therapy showed different mutational patterns of the *p53* and c-*kit* genes, suggesting different clonal evolution of lymphoma between the left and right sides. Mutation at codon 13 of the K-*ras* gene was detected in one of 14 cases (7.1%), and in exon 3 of the β-catenin gene in three of 12 cases (25%). All but one mutation were transition mutations, indicating that some endogenous mutagens act in lymphomagenesis in the adrenal gland. Our results suggest that *p53* and c-*kit* gene mutations might play a role in adrenal lymphomagenesis .**

Key words: Adrenal lymphoma — *p53* — c-*kit* — K-*ras* — β-Catenin

The adrenal gland is involved during the course of non-Hodgkin's lymphomas (NHL) in approximately one-fourth of cases.[1)] However, initial manifestation of NHL in the adrenal gland is extremely rare.[2)] In such cases, patients usually present with nonspecific signs and symptoms, such as fever, fatigue, and weight loss. Physical and roentgenographic examinations usually reveal huge and bilateral adrenal masses without lymphadenopathy, accompanied by adrenal insufficiency in some cases.[2)] Prognosis is very poor compared to other types of extranodal NHL. Histologic and immunohistochemical studies show that most of cases are diffuse large B-cell lymphomas.[2)] Adrenal lymphoma is one of the Epstein-Barr virus (EBV)-associated lymphomas: EBV genome was detected in 45% of the cases with occasional expression of latent membrane protein-1.[3)]

Accumulation of gene mutations results in development of malignant tumors, including NHL. In this study, the mutations of the *p53*, c-*kit*, K-*ras*, and β-catenin genes were examined in 17 cases of NHL with initial manifestation in the adrenal gland.

The *p53* gene is a well-known tumor suppressor gene that causes cell cycle arrest at the $G_1$ phase or stimulates expression of the *bax* gene, the protein that promotes apoptosis in cells with damaged DNA.[4)] In a wide variety of human cancers, *p53* gene mutations have been detected mainly in exons 5 through 8.[5)] High incidence of malignant lymphoma in *p53* knockout mice has been reported,[6)] suggesting an important role of *p53* gene mutations in lymphomagenesis.

The c-*kit* gene encodes a receptor tyrosine kinase, and the signal transduction mediated by c-*kit* receptor tyrosine kinase (*KIT*) plays a crucial role in proliferation and differentiation of hematopoietic stem cells, mast cells, and interstitial cells of Cajal.[7, 8)] Activating mutation of *KIT* (Asp816→Val) in the kinase domain of the c-*kit* gene was described in mast cell disorders.[9)] The development of acute leukemia or malignant lymphoma was also reported in transgenic mice expressing a *KIT* mutant (Asp816→Val).[10)] Recently, a relative high frequency of c-*kit* gene mutations in human nasal NK/T-cell lymphoma was reported.[11)]

The K-*ras* gene encodes a 21-kD ras protein, GTP- and GDP-binding protein, which plays a role in signal transduction through transmembrane signaling systems.[12)] K-*ras* mutations are frequently observed in pancreatic, colorectal, and lung adenocarcinomas,[12)] but rarely in the ordinary type of NHL.[13)] Thyroid lymphoma, a lymphoma which develops in autoimmune thyroiditis, showed relatively frequent mutations of the K-*ras* gene (25% of cases).[14)] The mutations tended to accumulate in high-grade B-cell lymphoma with replication error phenotype,

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suggesting a possible association of alterations of the K-*ras* gene with a subset of high-grade B-cell lymphomas. Because the majority of adrenal lymphoma was high-grade B-cell lymphoma, we examined K-*ras* mutations in the present study.

β-Catenin is associated with E-cadherin-mediated cell-cell adhesion and acts downstream of the Wnt signaling pathway.[15, 16] Activating mutations of the β-catenin gene at a crucial regulatory site in exon 3 result in accumulation of β-catenin protein in the cytoplasm.[17] The activated β-catenin complex leads to overexpression of c-*myc*,[18] which is related to cell proliferation, thus causing tumorigenesis. In fact, mutations of the β-catenin gene were reported in cancers of the colon,[19] endometrium,[20] and liver.[21] Although there have been no reports describing mutations of the β-catenin gene in malignant lymphomas, Knowles *et al.* reported a crucial role of overexpression of the c-*myc* gene in the development of EBV-associated lymphoproliferative disorders.[22] Because adrenal lymphoma is occasionally EBV-associated, we examined β-catenin gene mutations.

## MATERIALS AND METHODS

**Case selection** Seventeen cases of NHL with initial manifestation in the adrenal gland were selected for this study through a review of the "Annual of the Pathological Autopsy Cases in Japan (1992–1996)" (11 cases) and the Japanese medical journals (six cases). Clinical findings of these cases are summarized in Table I. There were 13 males and four females with ages ranging from 25 to 84 (median 68) years. They were admitted to hospitals during 1985 to 1996. The histologic diagnosis of adrenal NHL was made by biopsy (eight cases), surgery (two cases), or autopsy (seven cases). Bilateral adrenal glands were involved at presentation in all but two cases (cases 6, 16). In all cases, the main masses were located in the adrenal region, and the clinical stage was I in one case, II in 11, and III in two. Mediastinal mass was not noted in any patient during the clinical course. Endocrinological abnormalities such as serum adrenocorticotropic hormone, serum renin, urine noradrenaline, dopamine, low serum aldosterone, and/or lack of responsiveness to rapid adrenocorticotropic hormone test were found in eight of the 11 cases evaluated. In two cases (cases 8 and 10), samples from adrenal glands on both sides were examined (Table II). Metastatic lesions of lymph nodes and liver were also examined in cases 5 and 12, respectively. Histologic specimens were fixed in 10% buffered or neutral formalin, and routinely processed for paraffin-embedding. Histologic sections, cut at 3 μm, were stained with hematoxylin-eosin and by means of immunohistochemical procedures. Sources of samples for molecular study are shown in Table II. Biopsy and surgery were performed prior to chemotherapy and/or radiotherapy. Among 12 cases in which autopsy materials were used for analysis, seven received chemotherapy or radiation, but five did not receive any adjuvant therapy.

**Immunohistochemistry** Immunohistochemical study on the paraffin-embedded sections was carried out using the avidin-biotin-peroxidase complex method. Primary antibodies used in the study, suppliers and dilutions were as follows: CD3 (Dakopatts, Glostrup, Denmark; 1:100), CD43 (Bioscience, Emmenbrucke, Switzerland; 1:50), CD20 (Kyowa Medex, Tokyo; 1:200), CD45RO (Dakopatts; 1:100), and MB-1 (Bioscience; 1:50). Sections were treated with 0.1% trypsin solution (Sigma, St. Louis, MO) at 37°C for 30 min before reaction with anti-CD3. Histologic sections were reviewed by two of the authors (S. N. and K. A.), and lymphomas were classified based on the Revised European-American classification of lymphoid neoplasms.[23] The alkaline phosphatase-anti-alkaline-phosphatase method was used in p53 protein detection with monoclonal anti-human p53 protein (DO-7) (Dakopatts) diluted at 1:10 as the primary antibody. When DO-7 was used as the primary antibody, sections in 10 m*M* citrate buffer (10 m*M* citrate monohydrate in distilled water, pH 6.0) were treated with a microwave oven for 15 min for antigen retrieval. Cases with more than 10% of tumor cells positive for DO-7 were regarded as positive.

**Detection of *p53*, K-*ras*, c-*kit*, and β-catenin gene mutations** DNA for PCR amplification was extracted from paraffin sections using chelating resin. Selected exons in each gene, representing hot spots, were analyzed in this study. Sequences of the PCR primer pairs for the amplification of exon 5 of *p53* gene were 5′-TCTGTCTCCTTC-CTCTTCCTA-3′ and 5′-CATGTGCTGTGACTGCTTGT-3′ for the upper half region, and 5′-TGTGCAGCT-GTGGGTTGATTC-3′ and 5′-CAGCCCTGTCGTCTCTC-CAG-3′ for the lower half. Primer sequences for exons 6 through 8 of the *p53* gene, exon 1 of the K-*ras* gene, exons 11 and 17 of the c-*kit* gene, and exon 3 of the β-catenin gene were described previously.[11, 14, 24] DNA amplification and non-radioactive single strand conformation polymorphism (Cold SSCP) analyses were carried out to detect mutations as described previously.[25] The mutated bands at SSCP were extracted from the gel and reamplified by polymerase chain reaction (PCR) for 25 cycles to enrich mutated alleles.

Sequencing was performed by the dideoxy chain termination method using the Big Dye terminator cycle sequencing kit (Perkin-Elmer, Foster City, CA). Sequencing primers were the same as those used for PCR. Samples were analyzed with a Genetic Analyzer (ABI PRISM 310; Perkin-Elmer). PCR-SSCP analyses and sequencing of mutated bands were repeated three times for each sample to rule out the possibility of contamination and PCR fidelity artifacts.

## RESULTS

**Histological and immunohistochemical findings** Histologically all cases showed a diffuse proliferation of large lymphoid cells of predominantly noncleaved cell morphology, with highly pleomorphic cells in one case (case 5). Immunohistochemically, 15 cases were CD20+, MB-1+/−, CD43−/+, CD45RO−/+, CD3−; they were judged as diffuse large B-cell lymphomas. Two cases (cases 6 and 12) were CD20−, MB-1−, CD43+/−, CD45RO+, CD3+, and thus were judged as peripheral T-cell lymphomas, not specified (Table II). Tumor cells invaded the surrounding fat tissue, frequently yielding necrosis and fibrosis, often with hyalinization. Prominent intravascular proliferation of tumor cells in the adrenal gland and other organs was found in nine cases (Fig. 1). Positive immunoreactivity for DO-7 was found in seven of 21 (33.3%) lesions examined.

**Mutations of *p53*, c-*kit*, K-*ras*, and β-catenin gene** Results of gene mutation analyses are summarized in Table II, and the electrophoretic patterns of representative cases are illustrated in Fig. 2. Not all genes/exons could be studied due to failure of PCR in some target fragments of genes, which might be caused by artifacts from sample processing. By the direct sequencing of SSCP products, 19 single-nucleotide substitution mutations of *p53* gene were detected in nine of 17 cases (52.9%); 17 mutations (eight cases) at exon 5 and two mutations (two cases) at exon 7. In cases 3, 9, 10 (left gland), and 11, two or more mutations in exon 5 were detected in the extracts from aberrant bands on SSCP electrophoresis. In that case, direct sequencing of the full length of the products was performed in the same experiment. This indicated that these multiple mutations were present in the same allele. Cases 8 and 10 showed different patterns of mutations between

Table I. Clinical Findings in 17 Cases of Adrenal Lymphoma

| Case No. | Age | Sex | Presenting symptoms | Endocrinological abnormality[a)] | Adrenal mass | Size (cm) | Stage | Treatment[b)] | Outcome[c)] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 59 | M | Pain, hematuria | + | Bilateral | 11×7 | III | Chemotherapy (regimen: unknown), radiation | 57AT |
| 2 | 42 | M | None | NA | Bilateral | 13×11 | II | Chemotherapy (CHOP, M-BACOD, CDDP+VP16+PSL, AraC) | 9DT |
| 3 | 68 | M | Fever | NA | Bilateral | 12.5×10 | II | Chemotherapy (CDDP+VP16+VCR) | 5DT |
| 4 | 72 | F | Abdominal pain | − | Bilateral | 5×5 | II | Chemotherapy (CHOP, MEPP) | 8DT |
| 5 | 57 | M | Fever, pigmentation | + | Bilateral | NA | II | Chemotherapy (COP-B, CHOP-B, MACOP-B) | 12DID |
| 6 | 67 | M | None | + | Left side | 11×11 | NA | Operation | 10NED |
| 7 | 76 | F | General fatigue, dulness of lower limbs | + | Bilateral | 1.1 | NA | None | 4DID |
| 8 | 71 | M | Fever, apetite loss | NA | Bilateral | NA | II | Chemotherapy (CHOP) | 9DID |
| 9 | 84 | M | Fever, apetite loss | NA | Bilateral | NA | II | Chemotherapy (THP-COP) | 10DT |
| 10 | 80 | M | Fever, apetite loss | + | Bilateral | 8×4 | II | None | 1DID |
| 11 | 74 | M | Fever | NA | Bilateral | NA | II | Radiation | 4D |
| 12 | 74 | M | General fatigue, fever | NA | NA | NA | II | None | 3DT |
| 13 | 63 | M | Fever, pigmentation | + | Bilateral | 6×4.5 | II | Chemotherapy (regimen: unknown) | 3AT |
| 14 | 69 | F | Fever | − | Bilateral | 1.3 | NA | None | 1DT |
| 15 | 65 | M | Abdominal pain, pigmentation | + | Bilateral | 8×7 | III | Operation, chemotherapy (THP-COP), radiation | 3A |
| 16 | 25 | F | Fever | − | Left side | 19×12 | I | Operation, chemotherapy (ADM+EDX+VDS+PSL, ADM+MTX+VP16+PSL) | 10NED |
| 17 | 55 | M | Abdominal pain, apetite loss, weight loss | + | Bilateral | 12×8 | II | None | 2DID |

*a*) High in serum adrenocorticotroptic hormone, serum renin, urine noradrenaline, dopamine, low in serum aldosterone, and no responsive to rapid adrenocorticotropic hormone test.
*b*) ADM, adriamycin; AraC, cytarabine; BLE, bleomycin; CDDP, cisplatinum; EDX, cyclophosphamide; MTX, methotrexate; PSL, prednisolone; VCR, vincristine; VDS, vindesine; VP16, etposide. CHOP: EDX, ADM, VCR, PSL. CHOP-B: CHOP+BLE. COP-B: CTX, VCR, PSL, BLE. MACOP-B: MTX, doxorubicin, CTX, VCR, PSL, BLE. M-BACOD: MTX, BLE, ADM, EDX, VCR, dexamethasone. MEPP: mitoxantrone, VP16, CDDP, PSL. THP-COP: therarubicin, CTX, VCR, PSL.
*c*) AT, alive with tumor; DID, death due to intercalated disease; DT, death due to tumor; NED, no evidence of disease; NA, information not available.

Table II. Mutations of *p53*, c-*kit*, K-*ras*, and β-Catenin Gene in 17 Cases of Adrenal Lymphoma

| Case No. | Histologic diagnosis | Samples for molecular study | | *p53* mutation | | | | DO-7 expression | c-*kit* mutation | | | | K-*ras* mutation | | | | β-Catenin gene mutation | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Site | Procedure | Exon | Codon | Nucleotide | Amino acid | | Exon | Codon | Nucleotide | Amino acid | Exon | Codon | Nucleotide | Amino acid | Exon | Codon | Nucleotide | Amino acid |
| 1 | DLBL | Adrenal gland | Biopsy | 5 | 168 | CAC→TAC | His→Tyr | − | — | | | | — | | | | — | | | |
| | | | | 7 | 247 | AAC→AAT | Asn→Asn | − | | | | | | | | | | | | |
| 2 | DLBL | Adrenal gland | Autopsy | — | | | | | 11 | 566 | AAC→AAT | Asn→Asn | — | | | | | 32 | GAC→CAC | Asp→His |
| | | | | | | | | | 17 | 825 | GTT→GCT | Val→Ala | | | | | | | | |
| 3 | DLBL | Adrenal gland | Autopsy | 5 | 142 | CCT→TCT | Pro→Ser | + | 11 | 566 | AAC→AAT | Asn→Asn | — | | | | ND | | | |
| | | | | 5 | 151 | CCC→CTC | Pro→Leu | | 11 | 585 | CCC→CCT | Pro→Pro | | | | | | | | |
| 4 | DLBL | Adrenal gland | Autopsy | — | | | | − | 11 | 557 | TGG→CGG | Trp→Arg | 1 | 13 | GGC→GAC | Gly→Asp | — | | | |
| | | | | | | | | | 17 | 825 | GTT→GCT | Val→Ala | | | | | | | | |
| 5-1 | DLBL | Adrenal gland | Autopsy | — | | | | − | 11 | 557 | TGG→CGG | Trp→Arg | — | | | | — | | | |
| | | | | | | | | | 17 | 822 | AAT→AAC | Asn→Asn | | | | | | | | |
| | | | | | | | | | 17 | 825 | GTT→GCT | Val→Ala | | | | | | | | |
| -2 | DLBL | Adrenal gland | Autopsy | — | | | | − | — | | | | — | | | | — | | | |
| -3 | DLBL | Virchow LN | Biopsy | — | | | | + | — | | | | — | | | | — | | | |
| -4 | DLBL | Cervical LN | Autopsy | — | | | | − | 11 | 557 | TGG→CGG | Trp→Arg | — | | | | | 40 | ACT→ACC | Thr→Thr |
| | | | | | | | | | 17 | 825 | GTT→GCT | Val→Ala | | | | | | | | |
| 6 | PTCL | Adrenal gland | Surgery | — | | | | + | ND | | | | — | | | | ND | | | |
| 7 | DLBL | Adrenal gland | Autopsy | — | | | | − | 17 | 825 | GTT→GCT | Val→Ala | — | | | | ND | | | |
| 8-1 | DLBL | Adrenal gland (lt) | Autopsy | — | | | | ND | 17 | 825 | GTT→GCT | Val→Ala | — | | | | ND | | | |
| -2 | DLBL | Adrenal gland (rt) | Autopsy | 5 | 172 | GTT→GCT | Val→Ala | + | — | | | | — | | | | — | | | |
| 9 | DLBL | Adrenal gland | Autopsy | 5 | 133 | ATG→ACG | Met→Thr | − | 17 | 825 | GTT→GCT | Val→Ala | — | | | | — | | | |
| | | | | 5 | 144 | CAG→CGG | Gln→Arg | | | | | | | | | | | | | |
| | | | | 5 | 177 | CCC→CTC | Pro→Leu | | | | | | | | | | | | | |
| 10-1 | DLBL | Adrenal gland (rt) | Autopsy | 5 | 168 | CAC→CAT | His→His | − | 11 | 554 | GAA→GGA | Glu→Gly | ND | | | | ND | | | |
| | | | | | | | | | 11 | 559 | GTT→ATT | Val→Ile | | | | | | | | |
| | | | | | | | | | 17 | 825 | GTT→GCT | Val→Ala | | | | | | | | |
| -2 | DLBL | Adrenal gland (lt) | Autopsy | 5 | 133 | ATG→ACG | Met→Thr | − | 11 | 566 | AAC→GAC | Asn→Asp | ND | | | | — | | | |
| | | | | 5 | 144 | CAG→CGG | Gln→Arg | | | | | | | | | | | | | |
| | | | | 5 | 170[a)] | ACG→ACA | Thr→Thr | | | | | | | | | | | | | |
| | | | | 5 | 171 | GAG→GAA | Glu→Glu | | | | | | | | | | | | | |
| 11 | DLBL | Adrenal gland | Autopsy | 5 | 166 | TCA→TTA | Ser→Leu | − | — | | | | — | | | | — | | | |
| | | | | 5 | 177 | CCC→CCT | Pro→Pro | | | | | | | | | | | | | |
| 12-1 | PTCL | Adrenal gland | Autopsy | 5 | 172 | GTT→ATT | Val→Ile | + | 11 | 574 | ACA→ATA | Thr→Ile | — | | | | — | | | |
| | | | | 7 | 248[a)] | CGG→TGG | Arg→Trp | | | | | | | | | | | | | |
| -2 | PTCL | Liver | Autopsy | — | | | | + | — | | | | — | | | | — | | | |
| 13 | DLBL | Adrenal gland | Biopsy | — | | | | ND | ND | | | | ND | | | | ND | | | |
| 14 | DLBL | Adrenal gland | Autopsy | 5 | 172 | GTT→ATT | Val→Ile | − | 11 | 551 | CCC→TCC | Pro→Thr | — | | | | | 40 | ACT→ACC | Thr→Thr |
| | | | | | | | | | 11 | 557 | TGG→CGG | Trp→Arg | | | | | | | | |
| 15 | DLBL | Cervical LN | Biopsy | — | | | | + | — | | | | — | | | | — | | | |
| 16 | DLBL | Adrenal gland | Surgery | 5 | 177 | CCC→CTC | Pro→Leu | − | ND | | | | ND | | | | ND | | | |
| 17 | DLBL | Adrenal gland | Autopsy | — | | | | − | — | | | | — | | | | — | | | |

*a*) G:C to A:T transition at CpG dinucleotides site.
DLBL, diffuse large B-cell lymphoma; PTCL, peripheral T-cell lymphoma; LN, lymph node; lt, left; rt, right; ND, not done.

the left and right lesions. In case 10, a single mutation was found in the right adrenal gland and four in the left. Fourteen mutations were missense mutations leading to amino acid substitutions, and five were silent mutations resulting in no amino acid changes. G:C to A:T transitions were the predominant pattern of mutations (14 of 19 mutations, 73.7%), and the others were A:T to G:C transitions. Transversion mutations were never observed. Expression of p53

protein was found in four of 11 lesions without gene mutations and three of 10 with mutations.

As for the c-*kit* gene, 21 single-nucleotide substitution mutations were detected in 10 of 14 cases (71.4%); 12 mutations (seven cases) at exon 11 and nine mutations (seven cases) at exon 17. As found in the *p53* mutations, two mutations in exon 11 of cases 3, 10, and 14, and in exon 17 of case 5 (sample 5-1) were considered to occur in the same allele. Bilateral lesions in cases 8 and 10 showed different patterns of c-*kit* mutations, as observed in the case of *p53* mutations. Case 10 showed three mutations in the right adrenal gland and one in the left. Codon 825 was involved in seven cases, and both codons 557 and 566 were involved in three. Seventeen mutations were missense and four were silent. Six (28.6%) of 21 mutations were G:C to A:T transitions and the others were A:T to G:C transitions.

Mutation of the K-*ras* gene was detected only in one case (case 4) (7.1%). It was a G:C to A:T transition mutation at codon 13 of exon 1, and was a missense mutation (Gly→Asp). Three mutations of the β-catenin gene were detected in three of 12 cases (25.0%).

The frequencies of *p53*, c-*kit*, K-*ras*, and β-catenin gene mutations found in samples before chemotherapy or radiotherapy were 60%, 80%, 0%, and 25%, respectively, and those after therapies was 50%, 67%, 10%, and 25%. The frequencies before and after therapies were not significantly different.

## DISCUSSION

Recent reports have shown that *p53* mutations are associated with poor chemoresponsiveness and unfavorable prognosis in patients with aggressive B-cell lymphoma.[26] Döhner *et al.* reported that 17p deletion, possibly including the *p53* gene, was involved in the disease progression and affected the survival in chronic lymphocytic leukemia.[27] As for NHL, none of 43 cases in the United States[28] and eight of 48 (17%) in Japan[29] were reported to have *p53* mutations. In contrast, Lo Coco *et al.* reported that *p53* mutations were rather frequent (approximately 30%) in the aggressive type of B-cell NHL.[30] *p53* mutation is frequent in EBV-associated lymphomas; more than 50% in Burkitt's lymphoma,[31] 67% in pyothorax-associated lymphoma(PAL),[32] the lymphoma developing in the pleural cavity of patients with long-standing pyothorax, and 48% in nasal NK/T-cell lymphoma.[33] Adrenal lymphoma is also associated with EBV infection,[3] and in the

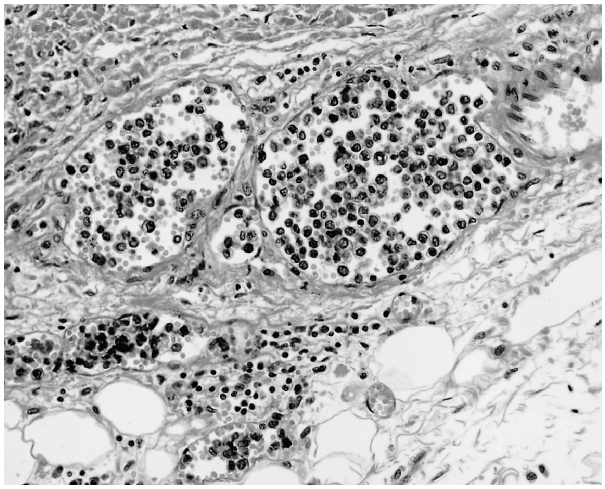

Fig. 1. Lymphoma cells show an intravascular proliferation in the connective tissue surrounding the adrenal gland (hematoxylin and eosin ×200).

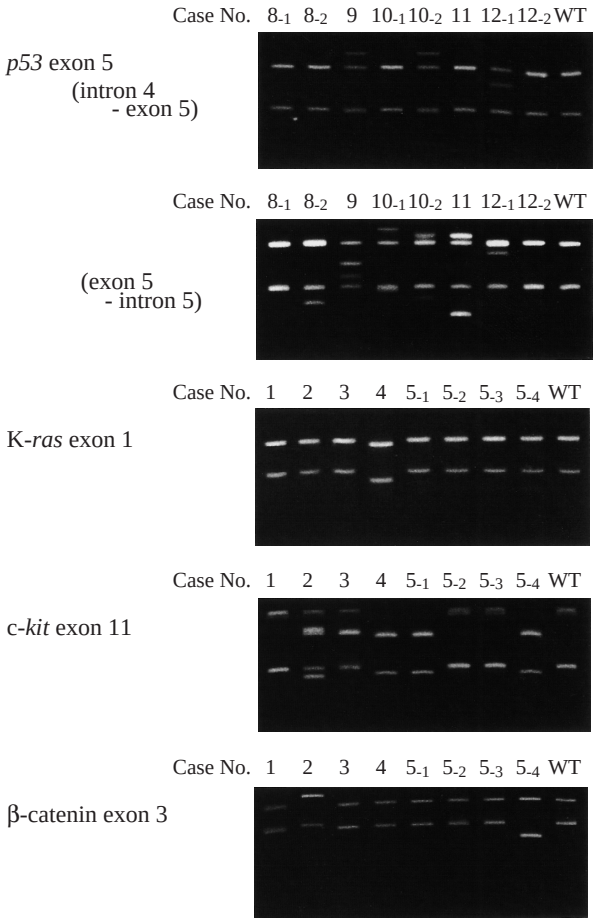


Fig. 2. "Cold" SSCP analysis of *p53*, K-*ras*, c-*kit*, and β-catenin genes in the tissues of the adrenal lymphoma and metastatic lesions from selected cases. Aberrant bands compared with wild-type (WT) suggested mutations, which were confirmed by direct sequencing. An aberrant band in exon 5 of *p53* gene (intron 4-exon 5) in sample 12-1 represents a mutation located in intron 4.

present study, we found a high frequency of *p53* mutations in adrenal lymphomas (nine of 17 cases, 52.9%), as in other EBV-associated lymphomas.

Predominant sites of *p53* mutations were not found in the previous studies on NHL,[34] but most of the mutations in adrenal lymphoma were observed in exon 5 (17 of 19, 89.5%). This was also observed in EBV-associated lymphomas, PAL[32] and nasal NK/T-cell lymphoma.[33] In case 12, mutation was found at codon 248, one of the so-called mutational "hot spots," [5] which involves an amino acid residue directly binding to DNA. Thus, this mutation might induce impairment of transcriptional activity of p53 protein. Mutations at codons 133, 142, 172, and 177 found in our cases were located at highly conserved regions in the core domain of the p53 protein, although whether these mutations alter the function of p53 protein is unclear. Restricted distribution of the mutations affecting codons 133, 144, 168, 172, and 177 might be a consequence of the selection of tumor clones with specific mutant alleles or an effect of specific mutagens.

As for the c-*kit* gene, the current study revealed a high frequency of mutations (10 of 14, 71.4%) in adrenal lymphoma. Previous reports showed that mutations at codon 816 in the kinase domain of the human the c-*kit* gene were frequently found in myelodysplastic disorders with mastocytosis[9] and most gastrointestinal stromal tumors had mutations within an 11 amino acid stretch (codon 550–560) in the juxtamembrane domain.[35] All these mutations proved to be gain-of-function mutations, because the cells transfected with these c-*kit* gene mutants show phospholyration of tyrosine of *KIT* and activation of *KIT* in the absence of stem cell factor, a ligand for *KIT*.[35, 36] In this study, 12 of 21 (57.1%) c-*kit* mutations were detected in exon 11, i.e., in the juxtamembrane domain. Eight of nine mutations in exon 17 were located at codon 825. Mutation at codon 825 was observed frequently in nasal NK/T-cell lymphoma,[11] but this c-*kit* mutant could induce neither phosphorylation of tyrosine nor activation of *KIT* in the absence of stem cell factor, and thus proved not to be a gain-of-function mutation.[11] Analyses for functional activity of other kinds of c-*kit* gene mutations in our cases were not performed.

Six cases in our series had both *p53* and c-*kit* gene mutations. Four of 10 cases (40.0%) with c-*kit* gene mutations did not have *p53* gene mutations. It was reported that c-*kit* signals inhibit *p53*-dependent apoptosis,[37] and thus c-*kit* mutations might play a role in oncogenesis in tumors without *p53* gene mutation. Bilateral adrenal lesions were examined in two cases (cases 8 and 10), showing different mutational patterns of *p53* and c-*kit* genes. Samples were obtained at autopsy in both cases. These were chosen because case 8 had received chemotherapy, which might induce a different mutational pattern, whereas case 10 did not receive any adjuvant therapy. The results suggest different clonal evolution of lymphoma between the left and right sides.

Except for thyroid lymphoma, mutation of *ras* genes in NHL is reported to be rare; none of 88 cases[13] and only three of 123 cases[38] were reported. Also in our cases, only one point mutation of the K-*ras* gene was observed in 14 cases examined.

There have been no comprehensive investigations on β-catenin gene mutations in malignant lymphomas. In this study three mutations of the β-catenin gene were found at exon 3, and two of these were silent mutations, indicating that involvement of the β-catenin gene in the development of adrenal lymphoma might be limited.

All of the mutations in *p53*, c-*kit*, K-*ras*, and β-catenin gene found in this study were transition mutations, with the exception of one mutation (β-catenin gene in case 2). As for *p53* gene mutations, G:C to A:T transitions were predominant (14 of 19 mutations, 73.7%), which is consistent with previous reports on lymphoid malignancies.[5] G:C to A:T transition is associated with endogenous oxidative or spontaneous deamination of 5-methylcytosine leading to replacement of cytosine by thymine. This replacement is not readily recognized by repair enzymes, thus resulting in G:C to A:T transition. These findings suggest that some endogenous mutagens act in adrenal lymphomagenesis.

Is the mutation frequency associated with any kind of treatment or not? Four of six cases without adjuvant therapy showed mutations in a gene, whereas one case receiving combined chemotherapy and radiation did not show any genetic abnormalities. In case 5, no genetic abnormalities were found in the lymph node lesion biopsied before treatment, whereas three transition mutations (T→C) were detected in the adrenal and lymph node lesions obtained from autopsy after adjuvant therapy. Myelodysplastic syndromes and acute myelogenous leukemia developing in patients treated with cyclophosphamide, an alkylating agent, preferentially showed single-base substitutions at A:T pairs in the *p53* gene.[39]

In conclusion, the *p53* and *c-kit* genes were frequently mutated in cases with adrenal lymphoma, suggesting that these genetic alterations might play an important role in lymphomagenesis. Adrenal lymphomas frequently show bilateral involvement, which might be a bifocal and biclonal development of lymphoma, but not a homing of lymphoma cells from one side to the other. Because selected exons in each gene, representing hot spots, were analyzed, it is not possible to draw any conclusions as to the mutation frequency of the genes investigated here.

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