Hypermethylation of death-associated protein (DAP) kinase CpG island is frequent not only in B-cell but also in T- and natural killer (NK)/T-cell malignancies

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(Received October 8, 2002/Accepted October 15, 2002)

Death-associated protein (DAP) kinase is a pro-apoptotic serine/threonine kinase with a death domain, which is involved in apoptosis induced by interferon-γ, tumor necrosis factor-α, and Fas ligand. Down-regulation of *DAP kinase* gene expression by hypermethylation of its promoter region might result in resistance to apoptotic cell death, and could provide a basis for tumor development. In the present study, we employed methylation-specific polymerase chain reaction to examine the methylation status of CpG islands in the *DAP kinase* gene in 19 cases of T-cell malignancies (including eight adult T-cell leukemia/lymphoma), 24 of natural killer (NK)/T-cell, and 34 of B-cell. Frequency of methylation was significantly higher in B-cell (27 of 34, 79.4%) than in T-cell malignancies (nine of 19, 47.4%) ($P<0.05$). Fifteen of 24 (62.5%) NK/T-cell lymphomas showed DNA methylation. One B-cell lymphoma cell line with DNA methylation was resistant to apoptotic stimuli, and treatment of the cells with a demethylating agent restored apoptotic cell death. These findings suggested that suppression of DAP kinase expression by DNA methylation might play a substantial role in the development of not only B-cell, but also T- and NK/T-cell lymphomas. (Cancer Sci 2003; 94: 87–91)

Apoptosis (programmed cell death) is an important regulatory mechanism for normal development and maintenance of the tissues in multicellular organisms. Several critical genes involved in apoptosis have been identified, such as *p53*, *bcl-2*, and *Bax*. Alterations of these genes have been described in various cancers, and are thought to result in accumulation of cells in which additional genetic changes occur, leading to tumor development.

Death-associated protein (*DAP*) *kinase* gene is one of the genes which functions as a positive mediator of interferon-γ-induced apoptosis.[1)] Death-associated protein kinase is a calcium/calmodulin-dependent serine/threonine kinase with a death domain,[2)] and is thought to play a role in an early apoptotic checkpoint designated to eliminate pre-malignant cells during cancer development.[3)] Overexpression of DAP kinase induces the death of HeLa cells without external stimuli, whereas a catalytically inactive mutant of this gene prevents interferon-γ-induced apoptosis in a dominant-negative manner.[4)] Recent studies demonstrated that DAP kinase also participates in tumor necrosis factor-α- and Fas-induced apoptosis.[5, 6)]

Cell lines derived from various human malignancies were reported to be resistant to interferon-γ-induced apoptosis as a result of diminished expression of DAP kinase at the mRNA or protein level.[7)] This down-regulation of DAP kinase expression has been found to be induced epigenetically by hypermethylation of the promoter region in the *DAP kinase* gene, which includes so-called CpG islands.[7, 8)] Hypermethylation of the *DAP kinase* gene was also found in varying frequencies in primary tumor samples, such as small cell lung cancer,[9)] head and neck cancers,[10)] and B-cell malignancies.[8)] We previously reported that hypermethylation was found in 84.2% of thyroid lymphomas, and suggested that the methylation of the *DAP kinase* gene plays a role in the development of thyroid lymphoma from chronic lymphocytic thyroiditis lesions.[11)] However, there has been no comprehensive study on the methylation state of T-cell and natural killer (NK)/T-cell lymphomas until now.

In this study, we analyzed the methylation state of CpG islands in the promoter region of the *DAP kinase* gene in 77 cases of malignant lymphomas, including 19 cases of T-cell, 24 of NK/T-cell, and 34 of B-cell lymphomas.

Materials and Methods

Case selection. Seventy-seven cases of lymphoid malignancies for which fresh frozen samples were available were selected for the present study: 62 cases from Osaka University, seven cases from our affiliated hospitals, and eight cases from Mie University. Fresh frozen tissue samples for methylation analysis and clinical information were collected from the patients after informed consents had been obtained. There were 49 males and 28 females with ages ranging from 20 to 75 (median 58.5) years. They received biopsy before chemotherapy from nodal (34 cases) and extranodal lesions (35 cases) during the period from 1987 to 2001. Peripheral blood lymphocytes were obtained from eight cases of adult T-cell leukemia/lymphoma (ATL). Histologic specimens were fixed in 10% formalin and routinely processed for paraffin-embedding. Histologic sections, cut at 5 μm, were stained with hematoxylin and eosin, and were reviewed by one of the authors (K. A.). All of the lymphomas were classified according to the Revised European American Lymphoma classification.[12)] The proportion of tumor cells among total cells in the field occupied by the tumor was estimated, and the cases were divided into three groups: high, ≥75%; intermediate, 25 to 75%; low, <25%.

Immunohistochemistry. Immunohistochemical study on paraffin-embedded sections was carried out using the avidin-biotin-peroxidase complex method. Primary antibodies used in the study, their 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), CD79a (Dakopatts; 1:50), TIA-1 (Coulter, Hialeah, FL; 1:500), MB-1 (Bioscience; 1:50), and CD56 (Zymed Laboratories, San Francisco, CA; 1:40). Sections were treated with 0.1% trypsin solution (Sigma, St. Louis, MO) at 37°C for 30 min before reaction with anti-CD3. When anti-CD79a and anti-CD56 antibodies were used as a primary antibody, sections rinsed in 10 m*M* citrate buffer (10 m*M* citrate

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monohydrate in distilled water, pH 6.0) were treated with a microwave oven for 5 min for antigen retrieval.

Cell culture. Two cell lines, OPL-1 and OPL-2, have been established from biopsy specimens of two cases of diffuse large B-cell lymphomas, which developed in the pleural cavity of patients with long-standing pyothorax, i.e. pyothorax-associated lymphoma (PAL).[13] The characteristics of these cell lines were reported previously.[13] OPL-1, -2, Raji, and Jurkat were cultured in RPMI1640 medium (Nissui, Tokyo) supplemented with 10% heat-inactivated fetal calf serum (BioWhittaker, Walkersville, MD), 100 U/ml penicillin, 100 μg/ml streptomycin, and 1 m*M* glutamine, in a humidified atmosphere with 95% air, and 5% CO_2 at 37°C. For demethylation of the CpG islands of the DNA, cells were treated with 0.5 μM 5-aza-2′-deoxycytidine for 6 days with a medium change on day 2. For induction of apoptosis, cells were treated with 0.15 μg/ml anti-Fas antibody (MBL, Nagoya).

Apoptosis analysis. Annexin V binds to phosphatidylserine and can be used to detect the loss of asymmetry in cell phospholipid, which is an event that appears during the early phase of apoptosis. Cells (5×10^5) were washed with binding buffer and subsequently incubated with ice-cold binding buffer containing annexin V-enhanced green fluorescent protein (EGFP) solution for 30 min in the dark. Without washing, the fluorescence of individual cells was measured by flow cytometry with a FACScan (Becton Dickinson Immunocytometry Systems, San Jose, CA). At least 30 000 events were collected for each specimen. The obtained data were analyzed using the CELL QUEST software (Becton Dickinson Immunocytometry Systems).

DNA extraction. Fresh frozen tissues obtained by surgical resection or open biopsy were stored at −80°C until use. Genomic DNA was extracted from frozen tissues, peripheral blood lymphocytes in cases of ATL, and the cell lines by a standard phenol/chloroform procedure.

Methylation-specific PCR (MSP). For MSP, we modified DNA with sodium bisulfite to convert all unmethylated cytosines, but not methylated cytosines, to uracils and we subsequently amplified the DNA with primers designed to distinguish the methylated from the unmethylated sequences in bisulfite-modified DNA. Bisulfite modification was performed as previously described[14] with some modification. Briefly, 1 μg of genomic DNA was denatured in 0.5 *M* NaOH and modified with 10 m*M* hydroquinone (Sigma) and 3 *M* sodium bisulfite (Sigma). Modified DNA was purified using the Wizard DNA Clean-up system (Promega, Madison, WI), treated with 0.3 *M* NaOH, precipitated with ethanol, and resuspended in 20 μl of water. PCR was carried out with primer pairs specific for the bisulfite-converted methylated or unmethylated promoter region of the *DAP kinase* gene[8] under the following conditions: 95°C for 10 min followed by 40 cycles at 95°C for 1 min, 60°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were run on the 3% NuSieve agarose gels, stained with ethidium bromide, and observed under UV illumination. DNA extracted from Raji was used as the methylated control. DNA extracted from Jurkat, samples biopsied from three cases with reactive lymphadenitis, and peripheral blood lymphocytes from three healthy volunteers were used as the unmethylated controls.

Total RNA extraction and RT-PCR for DAP kinase. Total RNA was extracted from the cell lines before and after demethylating treatment using Trizol reagent (Life Technologies, Rockville, MD). Total RNA could be extracted from biopsied samples of seven cases with methylated and one with unmethylated *DAP kinase* gene. For the RT-PCR, 1 μg of mRNA was converted to cDNA with 200 U of Molony murine leukemia virus reverse transcriptase (GIBCO-BRL, Gaithersburg, MD). The cDNA (1 μl of 10× diluted RT mixture) was amplified with the following primers for DAP kinase (5′-GAT AGA AAT GTC CCC AAA CCT CG-3′ and 5′-TCT TCT TTG GAT CCT TGA CCA GAA-3′) and for actin (5′-ATC ATG TTT GAG ACC TTC AA-3′ and 5′-CAT CTC TTG CTC GAA GTC CA-3′).[8] The conditions of amplification were as follows: 40 cycles at 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min for DAP kinase, and 30 cycles at 95°C for 30 s, 58°C for 1 min, and 72°C for 1 min for actin. The products were electrophoresed on the 1.5% NuSieve agarose gels.

DNA sequencing. A 5408 bp region (317 to 5724 from Genbank X76104) of the *DAP kinase* gene in OPL-1 and -2 was amplified by PCR using 12 sets of primers. cDNA were used as templates for amplification. The lengths of amplified fragments ranged from 132 to 611 bp. Amplified fragments were extracted from the gel and directly sequenced by the dideoxy chain termination method using a DNA sequencing kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions.

Statistical analysis. Fisher's exact test was performed to evaluate the significance of differences in frequency of methylation between subtypes of malignant lymphoma. The χ^2 test was used for evaluation of the significance of differences in proportions of tumor cells among total cells in the same field between methylated cases and unmethylated cases.

Results

Histological findings. Immunohistochemical analysis revealed that 34 cases were B-cell lymphomas, 19 T-cell lymphomas, and 24 NK/T-cell lymphomas (Table 1). B-Cell lymphomas included 17 diffuse large B-cell lymphomas, nine follicle center cell lymphomas, three marginal zone B-cell lymphomas, three mantle cell lymphomas, and one each of lymphoplasmacytoid lymphoma and plasmacytoma. Six of the diffuse large B-cell lymphomas were PAL. T-Cell lymphomas included eight ATL, nine peripheral T-cell lymphomas, unspecified, one angioimmunoblastic T-cell lymphoma, and one precursor T-lymphoblastic lymphoma. All ATL cases were seropositive for anti-human T-cell leukemia virus type 1 antibody. All but one of the NK/T-cell lymphomas ($CD56^+$, TIA-1^+) arose in the sino-nasal cavity.

MSP for *DAP kinase* gene promoter region. To confirm the efficacy of the procedure, MSP was performed on two control cell lines with proven methylation status of the *DAP kinase* gene.[8] A Burkitt's lymphoma cell line, Raji, showed amplification in the reaction for methylated sequences, but not for unmethylated ones. A T-cell leukemia cell line, Jurkat, showed amplification only for the unmethylated sequences (Fig. 1A). Because all samples from three cases with reactive lymphadenitis and peripheral blood lymphocytes from three healthy volunteers showed amplification only for the unmethylated sequences, electrophoretic patterns on MSP were judged on the all-or-none basis, i.e. positive or negative.

The results of MSP are summarized in Table 1, and representative cases are illustrated in Fig. 1. Fifty-one of 77 cases (66.2%) showed hypermethylation of the *DAP kinase* gene promoter region. As shown in Fig. 1, all cases, exhibited bands in the unmethylated reaction. These unmethylated bands might originate from non-neoplastic intermingling inflammatory cells, heterogeneity of the tumor cell population, or the unmethylated allele of the tumor cells. There are no significant differences in the proportions of tumor cells among total cells between methylated cases (high, 60%; intermediate, 33%; low, 7%) and unmethylated cases (high, 71%; intermediate, 29%; low, 0%). Frequency of the cases with hypermethylation of the *DAP kinase* gene in B-cell lymphomas (27 of 34 cases; 79.4%) was significantly higher than that in T-cell lymphomas (nine of 19 cases; 47.4%) ($P<0.05$) (Table 1). Fifteen of 24 cases (62.5%) with NK/T-cell lymphoma had DNA methylation. There are

Table 1. Incidence of methylation of *DAP kinase* gene according to histological subtypes

Histology	Number	Methylation	
B-Cell lymphoma	34	27/34 (79.4%)	
Diffuse large B-cell lymphoma (including PAL[1)])	17	13/17	
Follicle center cell lymphoma	9	8/9	
Marginal zone B-cell lymphoma	3	2/3	
Mantle cell lymphoma	3	3/3	P=0.019
Lymphoplasmacytoid lymphoma	1	0/1	
Plasmacytoma	1	1/1	
T-Cell lymphoma	19	9/19 (47.4%)	
Adult T-cell leukemia/lymphoma	8	3/8	
Peripheral T-cell lymphoma, unspecified	9	5/9	
Precursor T-lymphoblastic lymphoma	1	1/1	
Angioimmunoblastic T-cell lymphoma	1	0/1	
NK/T-Cell lymphoma	24	15/24 (62.5%)	
Total	77	51/77 (66.2%)	

1) PAL: pyothorax-associated lymphoma.

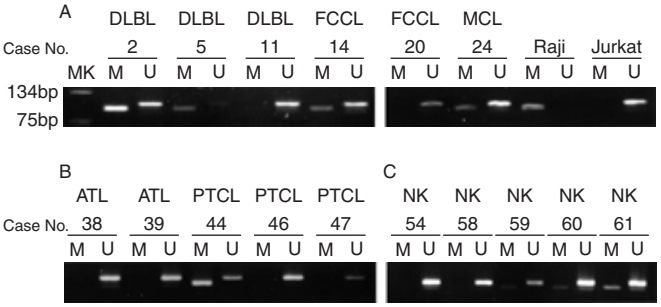

Fig. 1. Results of MSP for methylation status of DAP kinase promoter region. "M" and "U" indicate reactions using primers specific for methylated and unmethylated sequences, respectively. The sizes of MSP products for the methylated and unmethylated sequences are 98 bp and 106 bp, respectively. DLBL, diffuse large B-cell lymphoma; FCCL, follicle center cell lymphoma; MCL, mantle cell lymphoma; ATL, adult T-cell leukemia/lymphoma; PTCL, peripheral T-cell lymphoma, unspecified; NK, NK/T-cell lymphoma. (A) Results of MSP in the cell lines and B-cell malignancies. Raji shows amplification only in the methylated MSP product, whereas Jurkat does so only in the unmethylated MSP product. All cases, but not cell lines, show bands of the unmethylated product, because reactive cells are always admixed in the lesions. Cases 2, 5, 14, and 24 show bands of the methylated product. (B) Results in T-cell malignancies. One of the peripheral T-cell lymphomas (case 44) shows a band of the methylated product. (C) Three cases of NK/T-cell lymphomas (cases 59, 60, and 61) show bands of the methylated product.

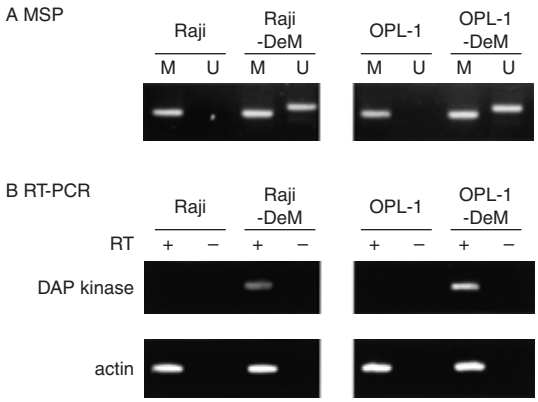

Fig. 2. Results of MSP and RT-PCR for DAP kinase in Raji and OPL-1 with or without demethylating treatment. (A) MSP shows that the *DAP kinase* gene is partially demethylated in both Raji and OPL-1 after treatment. (B) RT-PCR shows that the expression of DAP kinase mRNA is restored in both cell lines after demethylation. Raji-DeM and OPL-1-DeM indicate Raji and OPL-1 with demethylation, respectively. The sizes of RT-PCR products for DAP kinase and actin are 343 bp and 315 bp, respectively.

no significant differences in methylation frequency between NK/T-cell lymphomas and B-cell or T-cell lymphomas. Among B-cell lymphomas, four cases of diffuse large B-cell lymphomas and one case each of follicle center cell lymphoma, marginal zone B-cell lymphoma, and lymphoplasmacytoid lymphoma did not show hypermethylation. All three mantle cell lymphomas had methylation in the *DAP kinase* gene. Methylation was relatively infrequent in PALs (three of six cases; 50%). Among cases with T-cell lymphoma, ATLs showed a low frequency of methylation (three of eight; 37.5%). There was no significant difference in survival between the patients with (n=19) and without (n=8) methylation of the *DAP kinase* gene: the 5-year survival rates were 72.6% and 87.5%, respectively.

The *DAP kinase* gene was methylated in one (OPL-1) of two cell lines established from PAL cases (Fig. 2A), but not in the other (OPL-2) (data not shown). DNAs from primary tumor samples of PAL cases from which the OPLs had been derived were not available.

RT-PCR for *DAP kinase* gene expression. RT-PCR analysis showed no or a lower level of expression of DAP kinase mRNA in five of seven cases with a methylated promoter region (Fig. 3). A similar or rather lower level of expression was found in two cases with a methylated and one with an unmethylated gene. When compared with the control sample, a reactive lymphadenitis, two cell lines with methylation, Raji and OPL-1, also showed loss of DAP kinase mRNA expression (Fig. 2B).

Apoptosis analysis. For induction of apoptosis, Raji, Jurkat, OPL-1, and -2 were exposed to anti-Fas antibody. A majority of Jurkat cells showed apoptotic nuclear change, such as chromatin condensation and nuclear fragmentation. Flow-cytometric analysis showed a rightward shift of the peak of the fluorescence intensity of annexin V-EGFP after anti-Fas antibody treatment (Fig. 4A), indicating vulnerability of Jurkat cells to Fas-mediated apoptosis. Neither Raji nor OPL-1 with the methylated *DAP kinase* gene showed augmentation of the fluorescence intensity of annexin V-EGFP after anti-Fas antibody treatment (Fig. 4, B and C), indicating the resistance of both cell lines to anti-Fas antibody-induced apoptosis. OPL-2 did not show methylation in the *DAP kinase* gene in MSP, but the cells

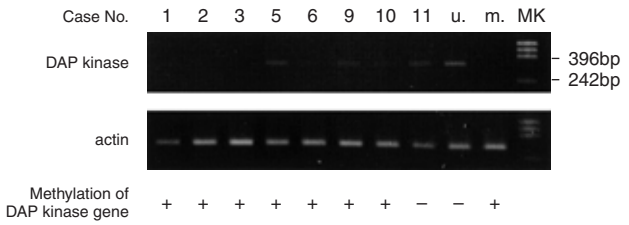

Fig. 3. Results of RT-PCR for DAP kinase mRNA in eight cases with diffuse large B-cell lymphoma. "u." indicates reactive lymphadenitis as an unmethylated control and "m." indicates Raji as a methylated control. Methylation status of each case is described below the electrophoregram. Five of seven cases with methylated promoter show loss of DAP kinase expression at the mRNA level.

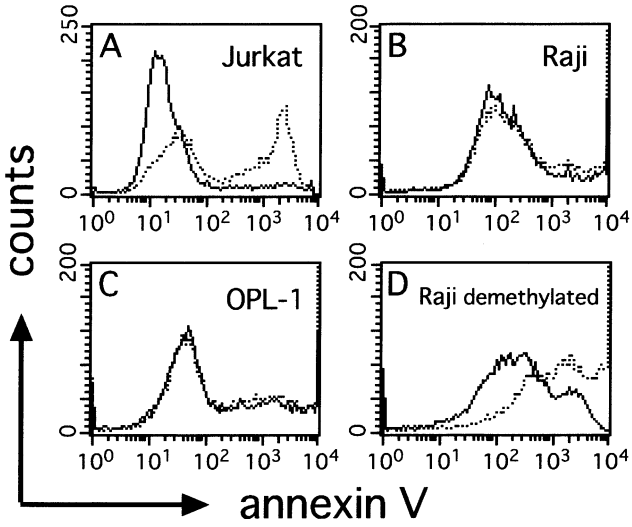

Fig. 4. Apoptosis induction by anti-Fas antibody in cell lines. Solid lines indicate the distribution of fluorescence intensities of the cells without anti-Fas antibody and dotted lines indicate that of the cells with anti-Fas antibody. A majority of Jurkat cells undergoes apoptosis (A), whereas Raji and OPL-1 do not (B, C). Raji after demethylating treatment shows partial apoptotic reaction (D).

were resistant to anti-Fas antibody-induced apoptosis (data not shown).

To determine whether aberrant methylation of the *DAP kinase* gene mediates the resistance of the cells to apoptosis, Raji and OPL-1 were treated with the demethylating agent, 5-aza-2′-deoxycytidine, and exposed to the anti-Fas antibody. The percentage of the cells showing apoptosis induction by anti-Fas antibody stimulation was increased after demethylating treatment in Raji (Fig. 4D), but not in OPL-1 (data not shown). MSP revealed that the *DAP kinase* gene CpG islands of both Raji and OPL-1 were partially demethylated after 5-aza-2′-deoxycytidine treatment (Fig. 2A), and reverse transcription PCR showed that DAP kinase mRNA expression was restored in both cell lines after demethylation (Fig. 2B).

DNA sequencing. Direct sequencing analysis did not showed any mutation within a 5408 bp region (317 to 5724 from Genbank X76104) of the *DAP kinase* gene in both OPL-1 and -2.

Discussion

Hypermethylation of the *DAP kinase* gene promoter region was reported in 23% of non-small-cell lung carcinoma[9] and in 18% of head and neck cancer.[10] Katzenellenbogen *et al.* examined the methylation of the *DAP kinase* gene in hematopoietic malignancies and normal lymphocytes.[8] Normal lymphocytes never showed methylation. However, 84% of B-cell lymphomas (although their histologic types and sites of origin were not described) in their series had methylated promoter regions of the *DAP kinase* gene. None of their T-cell lymphoma samples showed methylation, although only four samples were examined. In the present cases, the frequency of methylation in B-cell lymphomas (79%) was close to that in the report by Katzenellenbogen *et al*. In contrast to their series, however, nine of 19 cases (47%) with T-cell and 15 of 24 (63%) cases with NK/T-cell lymphomas showed methylation. Although the frequency of methylation in B-cell malignancies was significantly higher than that in T- and NK/T-cell malignancies, methylation of the *DAP kinase* gene might also play a role in T-cell malignancies.

The reason why the *DAP kinase* gene is frequently methylated in B-cell malignancies, but not in T-cell malignancies, is unknown. Several types of DNA methyltransferase (Dnmt1, Dnmt2, Dnmt3a, and Dnmt3b) have been identified in mammalian cells. Among them, Dnmt1 recognizes any target site for methylation on the whole genome and maintains the methylation pattern during DNA replication (maintenance methylation). In contrast, Dnmt3a and Dnmt3b are involved in *de novo* methylation in early development and are thought to play an important role in the formation of cell- or sequence-specific methylation patterns of the genome DNA.[15] These Dnmts with *de novo* methylating activity might also be involved in methylation acquired during carcinogenesis. It is possible that differences in the accessibility of the promoter region of the gene to these Dnmts and cell-specific expression pattern of Dnmts could account for the distinct patterns of DNA methylation among tumor cells with different phenotypes. On the other hand, another study reported that hypermethylation of CpG islands is not correlated with Dnmts expression, indicating that expression of Dnmts is not the only factor determining the methylation state of the genes.[16]

In the recent WHO classification for lymphoid neoplasms, NK/T-cell lymphomas stand as a distinct disease category, i.e., they are usually chemoresistant and show an aggressive clinical course, frequently Epstein-Barr virus-related, preferentially involve the nasal cavity, and histologically show an angiodestructive pattern of growth. Nasal NK/T-cell lymphoma is much more common in east Asians than in individuals of European background.[17] There have been no reports on the methylation state of *DAP kinase* gene in NK/T-cell lymphomas. The present study revealed that DNA methylation was found in a substantial proportion of NK/T-cell lymphomas of nasal type (62.5%), although there were no significant differences in methylation frequency between NK/T-cell and B-cell or T-cell lymphomas.

The RT-PCR analysis of DAP kinase mRNA in cell lines and primary tumor samples showed an association of methylation with suppression of DAP kinase mRNA expression, except for two cases. Addition of anti-Fas antibody induced apoptosis in Jurkat, a T-cell leukemia cell line with the unmethylated gene, but not in Raji, a B-cell lymphoma cell line with the methylated gene. Treatment of Raji with a demethylating agent restored the expression of DAP kinase mRNA and the cells became sensitive to apoptosis induced by anti-Fas antibody. These findings indicate that reduced expression of the DAP kinase caused by DNA methylation inhibits apoptosis of the cells. OPL-1, a cell line derived from PAL with a methylated *DAP kinase* gene, showed recovery of the expression of DAP kinase mRNA after demethylation, although responsiveness to apoptotic stimulation by anti-Fas antibody was not restored. Direct sequencing analysis did not show any mutation of the *DAP kinase* gene in OPL-1 and -2, indicating that other mechanisms, such as post-transcriptional modification and up-regulated degradation, are

involved in disruption of Fas-induced apoptosis in OPL-1. Alternatively, other factors than DAP kinase in the apoptosis induction pathway might suppress the apoptotic reaction. In fact, two of seven cases with methylated promoter retained mRNA expression of DAP kinase as compared with reactive lesion.

Disruption of apoptosis leads to prolonged survival of the lymphocytes, resulting in accumulation of gene abnormalities in the lesional lymphocytes, which might contribute to the development of lymphoma. We demonstrated a significantly higher frequency of methylation in thyroid lymphoma compared to chronic lymphocytic thyroiditis (84.2% versus 22.2%).[11] On the other hand, disruption of the Fas-Fas ligand apoptotic pathway through somatic mutation of the *Fas* gene is suggested to be involved in the development of several kinds of malignancies. However, a previous study revealed that the *Fas* gene mutation was detected at a relatively low frequency in non-Hodgkin's lymphoma,[18] suggesting that role of *Fas* gene mutation in lymphomagenesis is limited. Therefore it is supposed that suppression of apoptosis by diminished expression of DAP kinase might contribute to the development of lymphomas without *Fas* abnormalities. Indeed, a recent study showed that ectopic expression of DAP kinase suppressed oncogenic transformation of fibroblasts, through induction of apoptosis by activating *p53* in a *p19*-dependent manner.[3]

In conclusion, hypermethylation of the promoter region in the *DAP kinase* gene was frequent not only in B-cell malignancies, but also in T- and NK/T-cell malignancies, although the frequency was higher in the former than in the latter. Hematopoietic cell lines with a methylated *DAP kinase* gene showed resistance to apoptosis. In one B-cell line with methylated DNA, demethylating treatment restored the apoptotic reaction. These findings suggest that suppression of apoptosis of lymphocytes through DNA methylation provides a basis for lymphomagenesis, especially B-cell lymphomagenesis.

This work was supported in part by grants (12576004, 12670159, 12770087, 1270159) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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