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Microsatellite Instability and k-*ras*, *p53* Mutations in Thyroid Lymphoma

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Patho-epidemiological studies showed that thyroid lymphoma (TL) arises in inflammatory lesions of chronic lymphocytic thyroiditis (CLTH). Replication error (RER) is found in inflammatory lesions and associated cancer, suggesting that chronic inflammation could be a risk factor for neoplastic development through causing RER. To clarify whether RER is involved in the pathogenesis of TL, we examined the microsatellite instability (MSI) in 9 cases with CLTH and 19 with TL, including 10 diffuse large B-cell lymphoma (DLBL), 4 follicle center cell lymphoma, 3 marginal zone B-cell lymphoma of extranodal (MALT) type, and 2 lymphoplasmacytic type. Sixteen distinct microsatellite repeats were analyzed. Mutations of *p53* and k-*ras* genes were also examined. When alterations at 2 or more microsatellite loci were judged as positive, only 5 DLBL cases exhibited MSI. The frequency of MSI in DLBL was significantly higher than that in other types of TL and CLTH ($P<0.05$). Four of 19 cases (21.1%) showed point mutation of the k-*ras* gene. The k-*ras* mutations occurred in the cases with DLBL with RER, and four of five cases with RER had a k-*ras* mutation, indicating a close association between RER and k-*ras* mutation. *p53* mutations were not found in the CLTH. Two of 19 TL cases showed mutations of *p53* gene. There was no significant association between RER and *p53* mutation. These findings indicate that genomic instability contributes to the progression of TL from low grade to high grade, but not to the development of low grade lymphoma in CLTH lesions.

Key words: Chronic lymphocytic thyroiditis — Malignant lymphoma — Microsatellite instability — *p53* — k-*ras*

Thyroid lymphoma (TL) is a minor constituent of non-Hodgkin's lymphoma, accounting for 2.5% of all cases of extranodal lymphomas in the series of Freeman and associates from North America[1)] and 2.2% in our series from Japan.[2)] Several studies have suggested that TL originates from active lymphoid cells in autoimmune lymphocytic thyroiditis, i.e., Hashimoto's thyroiditis or chronic lymphocytic thyroiditis[3)] (CLTH). Follow-up studies confirmed an important role of CLTH in the development of TL.[4)] An immunophenotypic study revealed that TL are exclusively of B-cell origin.[5)] From patho-epidemiological studies on malignant lymphomas, we proposed that malignant lymphoma, exclusively of B-cell type, develops in chronic inflammation.[6)] Malignant lymphomas of the pleural cavity, urinary bladder, stomach, and thyroid developing in patients suffering from chronic pyothorax,[7)] chronic urocystitis,[8)] follicular gastritis caused by *Helicobacter pylori* infection,[9)] and CLTH[6)] are included in this concept. Reactive oxygen species released from polymorphonuclear leukocytes and macrophages cause DNA damage in the inflammatory lesions,[10)] and thus might contribute to tumorigenesis. Indeed, chronic inflammation appears to enhance tumorigenesis in lung, bowel, and skin.

Replication error (RER), as revealed by widespread microsatellite instability (MSI), is a manifestation of genomic instability caused by a defect in DNA mismatch repair function, and facilitates the fixation of genetic damage.[11)] Microsatellites are nucleotide repeat sequences which occur scattered throughout the genome. MSI has been detected in cancers associated with the hereditary nonpolyposis colorectal cancer syndrome, as well as in a variety of sporadic cancers, including gastric, endometrial, and colorectal cancers.[11)] MSI was reported in colonic mucosa with ulcerative colitis and associated carcinomas or in the parenchymal cells of pancreas affected by pancreatitis, suggesting that impaired DNA repair increases risk for the development of neoplasias in these patients.[12, 13)] Although MSI is uncommon in ordinary lymphomas,[14)] relatively frequent RER has been reported in gastric lymphoma or AIDS-related lymphoma. These findings suggest that infection and chronic inflammation could be risk factors for neoplastic development through causing RER.[15–17)]

In this study, we examined the MSI in TL and CLTH to clarify whether RER is involved in the pathogenesis of

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TL. Mutations of *p53* and k-*ras* genes were also examined by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) followed by direct sequencing, because *p53* and k-*ras* genes play both direct and indirect roles in maintaining genetic integrity, and mutations of these genes are commonly found in human malignancies.

MATERIALS AND METHODS

Cases Thyroid specimens were collected from 19 patients with TL and 9 with CLTH, who were admitted to the Kuma Hospital (Kobe) during the period 1995–98. All but 4 cases of TL were female. Age of patients on admission ranged from 45 to 70 (median 66) years in TL and 27 to 80 (median 67) years in CLTH. All patients underwent surgery, including total, partial thyroidectomy, or open biopsy. All of the histologic specimens were fixed in 10% formalin and routinely processed for paraffin-embedding or snap-frozen at −150°C and stored at −180°C until use. Criteria for the diagnosis of CLTH included increased consistency of the thyroid-gland, occasional hypothyroidism, high level of thyroid-stimulating hormone, low ^{123}I-uptake, and the presence of antimicrosomal and/or antithyroglobulin antibodies in the serum. Histologic findings of CLTH included lymphocytic infiltration, usually forming lymphoid follicles with germinal centers, varying degrees of fibrosis and oxyphilic change or squamous metaplasia in epithelial cells of the thyroid follicles. TL were classified according to the revised European-American classification for lymphoid neoplasms (REAL). Immunohistochemical study on paraffin sections from TL was carried out using the avidin-biotin-peroxidase complex method: monoclonal antibodies used as the primary antibody included L26 (CD20), CD3, UCHL-1 (CD45RO) (DAKO, Glostrup, Denmark), MB-1 and MT-1 (CD43) (Bioscience, Emmenbrucke, Switzerland).

Selection of microsatellite loci Sixteen distinct microsatellite repeats were analyzed by PCR in all cases (Table I). D3S1261, D3S1265, and c-myc have previously been shown to have a high frequency of instability in gastric lymphoma,[16, 17] as does D9S171 in non-Hodgkin's lymphoma of B-cell lineage.[18] BAT25 and BAT26, a mononucleotide repeat, were reported to show shortened alleles in human tumors with RER.[19] Three tumor suppressor loci (*p53*×2, DCC) were also selected. One of each primer pair was fluorescence-labeled with XRITC, HEX, NED or FITC dye.

Microsatellite analysis DNA was extracted from fresh-frozen thyroid tissues, as well as from peripheral blood leukocytes of the same patient, using the phenol-chloroform extraction method. A 15 μl aliquot of reaction mixture containing 100 ng of genomic DNA, 0.2 μM of each primer of the appropriate pair, 0.25 μM of each deoxynucleotide triphosphate, 1× PCR buffer, and 0.6 U of Ampli Taq Gold DNA polymerase (Applied Biosystems, Foster City, CA) was used for PCR. PCR conditions were as follows: 95°C for 10 min followed by 37 cycles (95°C for 30 s, 50°C for BAT25 and 26, 58°C for D3S643, 55°C for other markers, and 72°C for 30 s), and a final elongation at 72°C for 10 min. After amplification, reaction products (3 μl) were denatured and separated on 6% polyacrylamide gels containing 7 *M* urea. The gels were placed in an FMBIO-II (Takara, Kusatsu) and analyzed. The electrophoretic patterns of peripheral blood leukocytes and thyroid tissue from the same patient were then compared

Table I. Microsatellite Markers Used for MSI Analysis

Locus	Repeat	Location	Dye	Size (bp)	Source
D3S643	CA	3p21.3	XRITC	103	Takara
D3S1261	CA	3p12-14	XRITC	217	Takara
D3S1265	CA	3q27	XRITC	126–150	Takara
D6S309	CA	6p24-25	NED	307–333	Applied Biosystems
D9S171	CA	9p21	NED	164–188	Applied Biosystems
D11S1314	CA	11q13	HEX	97–123	Applied Biosystems
D14S74	CA	14p23	HEX	301–325	Applied Biosystems
D15S978	CA	15q11-13	HEX	187–215	Applied Biosystems
D22S539	CA	22q11	NED	203–221	Applied Biosystems
DCC	TA	18q21	FITC	150–210	Takara
MYC	CA	8q24	XRITC	113	Takara
P53(1)	CA	17p13	FITC	103–135	Takara
P53(2)	AAAAT	17p13	FITC	140–175	Takara
(AT)TSHR	AT	14q31	FITC	292–328	Takara
BAT25	Poly(A) tracts	4q12	XRITC	125	Takara
BAT26	Poly(A) tracts	2p21	FITC	121	Takara

Table II. Oligonucleotide Primers Used for PCR Reactions

k-*ras*		
	Exon 1	5′-CAT GTT CTA ATA TAG TCA CA-3′
		5′-CTC TAT TGT TGG ATC ATA TTC GTC C-3′
	Exon 2	5′-ACT GTG TTT CTC CCT TCT CA-3′
		5′-CAC AAA GAA AGC CCT CCC CA-3′
p53		
	Exon 5	5′-GTA CTC CCC TGC CCT CAA CA-3′
		5′-CTC ACC ATC GCT ATC TGA GCA-3′
	Exon 6	5′-TTG CTC TTA GGT CTG GCC CC-3′
		5′-CAG ACC TCA GGC GGC TCA TA-3'
	Exon 7	5′-TAG GTT GGC TCT GAC TGT ACC-3′
		5′-TGA CCT GGA GTC TTC CAG TGT-3′
	Exon 8	5′-AGT GGT AAT CTA CTG GGA CGG-3′
		5′-ACC TCG CTT AGT GCT CCC TG-3′

Table III. Microsatellite Alterations and Gene Mutations

Histology	No. of cases	Microsatellite alterations			Gene mutations	
		1 locus	≥2 loci		*p53*	k-*ras*
Chronic lymphocytic thyroiditis	9	2	0		0	0
Thyroid lymphoma						
Low-grade MALToma[a)]	9	5	0	[b)] $P<0.05$	2	0
Follicle center lymphoma						
Diffuse large B-celll lymphoma	10	1	5		0	4

a) Marginal zone lymphoma and lymphoplasmacytic lymphoma.
b) Fisher's exact test.

to detect different alleles caused by expansion or deletion of repeat tracts.

***p53* and k-*ras* mutations** Partially intron-based PCR primers of *p53* gene exons 5–8 and k-*ras* gene exons 1–2 are shown in Table II. PCR amplification and nonradioactive (cold) SSCP analysis were carried out to detect mutations as described previously.[20)] The aberrant SSCP bands were extracted from the gel and reamplified by PCR for 20 or 25 cycles to enrich the mutated alleles. Sequencing was performed by the dideoxy chain termination method using the Ampli Taq FS cycle-sequencing kit (Applied Biosystems). Sequencing primers were the same as those used for PCR. Cycle sequencing was performed following the usual protocol, i.e., 30 cycles of denaturation (95°C, 30 s), annealing (52°C, 30 s), and extension (72°C, 4 min) followed by 20°C after the final cycle. After ethanol precipitation, the samples were analyzed with a Genetic Analyzer (ABI PRISM 310′, Applied Biosystems). PCR-SSCP analysis and sequencing of mutated bands were repeated three times for each sample to rule out the possibility of contamination and PCR fidelity artifacts.

Statistical method Fisher's exact test was used to evaluate the significance of differences in the frequencies of MSI and mutations in *p53* and k-*ras* genes between CLTH and TL.

RESULTS

Histologic and immunohistologic findings Lymphoma cells in all cases showed a B-cell phenotype, i.e., $CD20^+$ and/or $MB\text{-}1^+$, $CD3^-$, $CD45RO^-$, $CD43^-$. The TL of this series were classified as diffuse large B-cell lymphoma (DLBL) in 10 cases, follicle center cell lymphoma in 4, marginal zone B-cell lymphoma of extranodal (MALT) type in 3, and lymphoplasmacytic type in 2.

Microsatellite alterations and RER The cases showing alterations at ≥2 microsatellite loci were judged as positive for MSI. Single-locus microsatellite changes in nontumor DNA are reported to be detectable at low frequency (1 to 4×10^{-3} per cell generation) in the absence of detectable defects in DNA mismatch repair,[21)] so such occasional microsatellite alterations were considered to be a back-

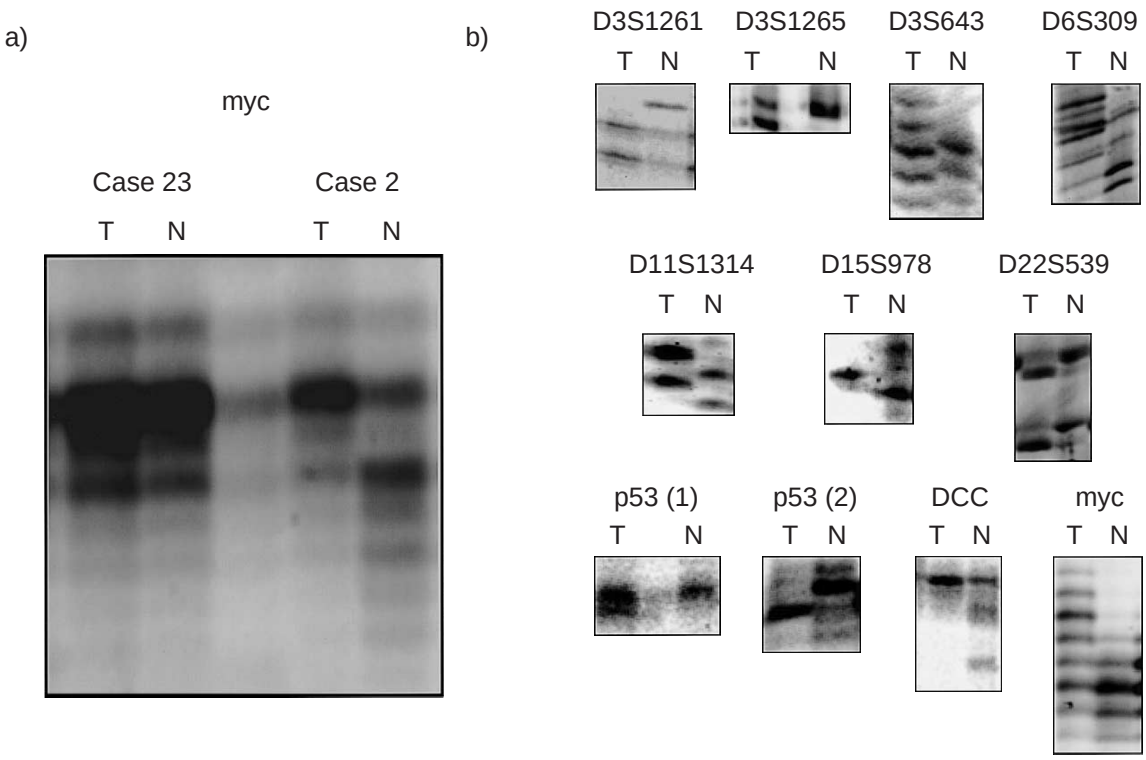

Fig. 1. Representative examples of microsatellite analysis in thyroid lymphomas (T) and peripheral blood cells (N) from the same patients. Sixteen distinct microsatellite repeats were analyzed as described in "Materials and Methods." a) Case 23 showed no microsatellite alteration, whereas Case 2 showed microsatellite alteration at the myc locus. b) D22S539 locus from marginal zone B-cell lymphoma of extranodal (MALT) type. Remaining figures were from cases of DLBL with RER. No cases showed alterations at D9S171, D14S74, BAT25 and BAT26 loci.

Table IV. Summary of Cases with Mutations of *p53* and k-*ras* Gene

Case	Age	Sex	Histology	RER	*p53*			k-*ras* exon 1[a)]	
					Exon	Codon	Nucleotide	Codon	Nucleotide
1	60	F	DLBL	8/16	—	—	—	12	GGT→GCT
2	61	F	DLBL	3/16	—	—	—	12	GGT→TGT
3	67	M	DLBL	2/16	—	—	—	13	GGC→GAC
4	80	F	DLBL	3/16	—	—	—	13	GGC→GAC
5	54	F	FCL	1/16	6	190	CCT→CTT	—	—
6	73	F	LP	1/16	8	272	GTT→GGT	—	—

a) No mutations were found in exon 2.
DLBL, diffuse large B-cell lymphoma; FCL, follicle center cell lymphoma; LP, lymphoplasmacytic lymphoma.

ground and not significant. RER is usually characterized by alterations in multiple loci,[22)] and this threshold would significantly lower the probability of regarding background mutations as positive.

The results are summarized in Table III and representative cases are illustrated in Fig. 1. Six cases of TL and 2 cases of CLTH showed alterations at a single microsatellite locus. Only 5 cases of DLBL exhibited alterations at ≥2 loci, i.e., 2 loci in 2 cases, 3 loci in 2 cases, and 8 loci in one case. The frequency of MSI in DLBL was significantly higher than that in other types of TL and CLTH ($P<0.05$). One DLBL case showed alterations at D3S1261

and D3S1265, and three cases at the c-myc locus. No cases exhibited shortened alleles at BAT25 and BAT26, which are indicators of RER in many human tumors.[19]

***p53* and k-*ras* mutations** Detected mutations of *p53* and k-*ras* genes are summarized in Table IV. Four of 19 cases (21.1%) had mutations at exon 1 of the k-*ras* gene: two were missense mutations in codon 13 and two were mutations in codon 12, one of which was a missense change and the other resulted in substitution of glycine (GGT) with cysteine (TGT). Two of the four substitutions were G:C→A:T, one was G:C→C:G, and one was G:C→T:A. CpG pairs were involved in none of the cases. None of the cases showed point mutations in exon 2 of the k-*ras* gene. It is noteworthy that the k-*ras* mutations occurred in the cases with DLBL with RER, and four of five cases with RER had a k-*ras* mutation. There is a significant association between RER and k-*ras* mutation ($P<0.05$).

p53 mutations were not found in the CLTH. Two of 19 TL cases (10.5%) showed mutations of the *p53* gene: one was a missense change in codon 190 at exon 6, and the other was a substitution mutation of valine (GTT) with glycine (TGT) in codon 272 at exon 8. One substitution was C:G→T:A and the other was T:A→G:C. CpG sites were not involved. No significant association between RER and *p53* mutation was found.

DISCUSSION

CLTH is an organ-specific autoimmune disease and predisposes to TL, with a delay of over 10 years until development of TL.[4] In this situation, MSI was suggested to occur in the thyroid lesions, because oxidative stress is responsible for as many as 10000 DNA-damaging events/cell/day under normal cellular conditions.[23] However, none of the present CLTH and TL of low grade cases showed MSI. Chronic inflammation in CLTH might intensify oxidative stress and induce DNA damage, but might be compensated by enhanced capacity of DNA repair.

Gastric lymphoma is now classified as MALT lymphoma,[24] and is considered to develop in *H. pylori*-induced chronic gastritis.[9] Chong *et al*.[16] reported that 66.7% of low-grade gastric MALT lymphomas showed RER. However, Xu *et al*.[25] have recently reported that none of 33 cases of primary gastric lymphoma of the MALT type exhibited RER, after examining 9 microsatellite loci including the same 7 microsatellite loci as selected by Chong *et al*.[16] These discrepancies might derive from the different criteria used to define RER: only one locus was enough for some studies and more than 2 loci in another. We regarded the cases showing alterations at ≥2 microsatellite loci as positive for MSI, and none of the 9 cases of primary TL of low grade or follicle center cell lymphoma exhibited RER, including alterations at D3S1261, D3S1265 and c-myc loci, which have been reported to have a high frequency of instability.[16] Thus, genomic instability might not contribute significantly to the molecular pathogenesis of low-grade MALT lymphoma developing in chronic inflammation.

In this study, 50% of DLBL exhibited RER, i.e., alterations at ≥2 loci. The frequency of MSI in DLBL was significantly higher than that in other types of TL and CLTH ($P<0.05$), suggesting that increase in the genomic instability contributes to the progression from low grade to high grade lymphoma of the thyroid. In gastric lymphoma, accelerated genomic instability in DLBL compared to low grade lymphomas was reported,[16, 17, 25] although the difference in MSI frequency was not statistically significant in the study by Xu *et al*.[25] Frequency of MSI in DLBL arising in the wall of chronic pyothorax, a type of lymphoma developing in chronic inflammation, was found to be about 30% (data not published). In nodal lymphomas, Gamberi *et al*.[14] found that MSI was not responsible for the clinical progression and histologic transformation, which are common in the late phase of nodal lymphomas. These findings might suggest different pathogenetic mechanisms for progression of nodal and extranodal lymphomas.

The k-*ras* gene-encoded protein, referred to as p21, functions as a G protein, which plays a role in signal transduction from growth factor receptors on the cell membrane. Point mutations in k-*ras* gene result in formation of proteins that can not be inactivated, thus leading to autonomous cell growth and proliferation. It was reported that leukocyte-derived potent oxidants could cause k-*ras* oncogene activation, which is believed to play a critical role in the pathogenesis of many human malignancies.[26] In our studies, a significant association between RER and k-*ras* mutation ($P<0.05$) was found; four of five DLBL with RER had k-*ras* mutation. MSI might lead to accumulation of genetic aberrations such as k-*ras* mutations, causing progression of low grade to high grade lymphoma. Alternatively, MSI may be present *per se* in some cases with DLBL. The association of RER with k-*ras* mutation was also reported in pancreatic cancer, non-small cell lung cancer, and a type of sarcoma in mice.[13, 27]

p53 is a well-known tumor suppressor gene, and has an anti-oncogenic role by causing cells with damaged DNA to arrest at the G1 phase of the cell cycle or stimulating expression of the *bax* gene, the protein product of which promotes apoptosis. *p53* mutation was involved in the development of splenic marginal zone lymphoma,[28] or associated with progression of MALT lymphoma.[29] In our cases, *p53* mutations were found in only 2 of 9 cases with low-grade MALT lymphoma, but in none of DLBL. These findings suggest that genomic instability does not always occur in concert with *p53* inactivation.

Free radicals cause DNA damage such as DNA deaminations and single-strand breaks. DNA deaminations

change DNA sequences as follows[30]; G:C→A:T, A:T→G:C, G:C→T:A, A:T→T:A. Indeed, in our cases, such changes were found in 3 of 4 cases with k-*ras* mutation, and in 1 of 2 cases with p53 mutations.

In conclusion, the present findings on TL indicate that the genomic instability contributes to the molecular pathogenesis of the progression from low grade to high grade lymphoma.

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REFERENCES

1) Freeman, C., Berg, J. W. and Cutler, S. J. Occurrence and prognosis of extranodal lymphomas. *Cancer*, **29**, 252–260 (1972).
2) Aozasa, K., Tsujimoto, M., Sakurai, M., Honda, M., Yamashita, K., Hanada, M. and Sugimoto, A. Non-Hodgkin's lymphomas in Osaka, Japan. *Eur. J. Cancer Clin. Oncol.*, **21**, 487–492 (1985).
3) Volpe, R. Thyroiditis: current views of pathogenesis. *Med. Clin. North Am.*, **59**, 1163–1175 (1975).
4) Kato, I., Tajima, K., Suchi, T., Aozasa, K., Matsuzuka, F., Kuma, K. and Tominaga, S. Chronic thyroiditis as a risk factor of B-cell lymphoma in the thyroid gland. *Jpn. J. Cancer Res.* (*Gann*), **76**, 1085–1090 (1985).
5) Aozasa, K., Ueda, T., Katagiri, S., Matsuzuka, F., Kuma, K. and Yonezawa, T. Immunologic and immunohistologic analysis of 27 cases with thyroid lymphomas. *Cancer*, **60**, 969–973 (1987).
6) Aozasa, K. Malignant lymphoma of the mucosa-associated lymphoid tissue. *Am. J. Surg. Pathol.*, **16**, 90–92 (1992).
7) Aozasa, K., Ohsawa, M. and Kanno, H. Pyothorax-associated lymphoma: a distinct type of lymphoma strongly associated with Epstein-Barr virus. *Adv. Anat. Pathol.*, **4**, 58–63 (1997).
8) Ohsawa, M., Aozasa, K., Horiuchi, K. and Kanamaru, A. Malignant lymphoma of bladder. Report of three cases and review of the literature. *Cancer*, **72**, 1969–1974 (1993).
9) Parsonnet, J., Hansen, S., Rodriguez, L., Gelb, A. B., Warnke, R. A., Jellum, E., Orentreich, N., Vogelman, J. H. and Friedman, G. D. *Helicobacter pylori* infection and gastric lymphoma. *N. Engl. J. Med.*, **330**, 1267–1271 (1994).
10) Cerutti, P. A. and Trump, B. F. Inflammation and oxidative stress in carcinogenesis. *Cancer Cells*, **3**, 1–7 (1991).
11) Eshleman, J. R. and Markowitz, S. D. Microsatellite instability in inherited and sporadic neoplasms. *Curr. Opin. Oncol.*, **7**, 83–89 (1995).
12) Suzuki, H., Harpaz, N., Tarmin, L., Yin, J., Jiang, H. Y., Bell, J. D., Hontanosas, M., Groisman, G. M., Abraham, J. M. and Meltzer, S. J. Microsatellite instability in ulcerative colitis-associated colorectal dysplasias and cancers. *Cancer Res.*, **54**, 4841–4844 (1994).
13) Brentnall, T. A., Chen, R., Lee, J. G., Kimmey, M. B., Bronner, M. P., Haggitt, R. C., Kowdley, K. V., Hecker, L. M. and Byrd, D. R. Microsatellite instability and K-ras mutations associated with pancreatic adenocarcinoma and pancreatitis. *Cancer Res.*, **55**, 4264–4267 (1995).
14) Gamberi, B., Gaidano, G., Parsa, N., Carbone, A., Roncella, S., Knowles, D. M., Louie, D. C., Shibata, D., Chaganti, R. S. and Dalla-Favera, R. Microsatellite instability is rare in B-cell non-Hodgkin's lymphomas. *Blood*, **89**, 975–979 (1997).
15) Bedi, G. C., Westra, W. H., Farzadegan, H., Pitha, P. M. and Sidransky, D. Microsatellite instability in primary neoplasms from HIV$^+$ patients. *Nat. Med.*, **1**, 65–68 (1995).
16) Chong, J. M., Fukayama, M., Hayashi, Y., Hishima, T., Funata, N., Koike, M., Matsuya, S., Konishi, M. and Miyaki, M. Microsatellite instability and loss of heterozygosity in gastric lymphoma. *Lab. Invest.*, **77**, 639–645 (1997).
17) Peng, H., Chen, G., Du, M., Singh, N., Isaacson, P. G. and Pan, L. Replication error phenotype and p53 gene mutation in lymphomas of mucosa-associated lymphoid tissue. *Am. J. Pathol.*, **148**, 643–648 (1996).
18) Fernandez, P. J., Santos, J., Perez, D. C. I., Melendez, B., Martinez, B., Robledo, M., Rivas, C. and Benitez, J. Frequent allelic losses of 9p21 markers and low incidence of mutations at p16 (CDKN2) gene in non-Hodgkin lymphomas of B-cell lineage. *Cancer Genet. Cytogenet.*, **98**, 63–68 (1997).
19) Zhou, X. P., Hoang, J. M., Li, Y. J., Seruca, R., Carneiro, F., Sobrinho, S. M., Lothe, R. A., Gleeson, C. M., Russell, S. E., Muzeau, F., Flejou, J. F., Hoang, X. K., Lidereau, R., Thomas, G. and Hamelin, R. Determination of the replication error phenotype in human tumors without the requirement for matching normal DNA by analysis of mononucleotide repeat microsatellites. *Genes Chromosom. Cancer*, **21**, 101–107 (1998).
20) Hongyo, T., Buzard, G. S., Calvert, R. J. and Weghorst, C. M. 'Cold SSCP': a simple, rapid and non-radioactive method for optimized single-strand conformation polymorphism analyses. *Nucleic Acids Res.*, **21**, 3637–3642 (1993).
21) Weber, J. L. and Wong, C. Mutation of human short tandem repeats. *Hum. Mol. Genet.*, **2**, 1123–1128 (1993).
22) Aaltonen, L. A., Peltomaki, P., Leach, F. S., Sistonen, P., Pylkkanen, L., Mecklin, J. P., Jarvinen, H., Powell, S. M., Jen, J., Hamilton, S. R., Peterson, G. M., Kinzler, K. W., Vogelstein, B. and de la Chapelle, A. Clues to the pathogenesis of familial colorectal cancer. *Science*, **260**, 812–816 (1993).
23) Fraga, C. G., Shigenaga, M. K., Park, J. W., Degan, P. and Ames, B. N. Oxidative damage to DNA during aging: 8-

hydroxy-2′-deoxyguanosine in rat organ DNA and urine. *Proc. Natl. Acad. Sci. USA*, **87**, 4533–4537 (1990).
24) Isaacson, P. G. and Spencer, J. Malignant lymphoma of mucosa-associated lymphoid tissue. *Histopathology*, **11**, 445–462 (1987).
25) Xu, W. S., Chan, A. C., Liang, R. and Srivastava, G. No evidence of replication error phenotype in primary gastric lymphoma of mucosa-associated lymphoid tissue. *Int. J. Cancer*, **76**, 635–638 (1998).
26) Jackson, J. H., Vollenweider, M., Hill, J., Rodriguez, H., Schwabacher, A. W., Mitra, G. and Kuo, C. Y. Stimulated human leukocytes cause activating mutations in the K-ras protooncogene. *Oncogene*, **14**, 2803–2808 (1997).
27) Niwa, O., Kamiya, K., Furihata, C., Nitta, Y., Wang, Z., Fan, Y. J., Ninomiya, Y., Kotomura, N., Numoto, M. and Kominami, R. Association of minisatellite instability with c-myc amplification and K-ras mutation in methylcholanthrene-induced mouse sarcomas. *Cancer Res.*, **55**, 5670–5676 (1995).
28) Baldini, L., Fracchiolla, N. S., Cro, L. M., Trecca, D., Romitti, L., Polli, E., Maiolo, A. T. and Neri, A. Frequent p53 gene involvement in splenic B-cell leukemia/lymphomas of possible marginal zone origin. *Blood*, **84**, 270–278 (1994).
29) Du, M., Peng, H., Singh, N., Isaacson, P. G. and Pan, L. The accumulation of p53 abnormalities is associated with progression of mucosa-associated lymphoid tissue lymphoma. *Blood*, **86**, 4587–4593 (1995).
30) Caulfield, J. L., Wishnok, J. S. and Tannenbaum, S. R. Nitric oxide-induced deamination of cytosine and guanine in deoxynucleosides and oligonucleotides. *J. Biol. Chem.*, **273**, 12689–12695 (1998).