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Frameshift Mutations and a Length Polymorphism in the *hMSH3* Gene and the Spectrum of Microsatellite Instability in Sporadic Colon Cancer

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Mutations in the *hMSH3* gene in sporadic colon cancer with microsatellite instability (MSI) were investigated, since several mismatch repair genes were known to be mutated in cancers with MSI, but only deletions in the $(A)_8$ region in the *hMSH3* gene have been reported. We also analyzed the relationships between *hMSH3* mutations and the spectrum of MSI. We screened MSI in 79 sporadic colon cancer samples using mono- and dinucleotide repeat markers and the samples with MSI were further analyzed for tri- and tetranucleotide repeat instability and mutations in the *hMSH3* gene by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis. Five (6%) out of 79 tumors were MSI-H and 15 (19%) were MSI-L. Two MSI-H tumors showed insertion in the $(C)_8$ region in the *hMSH6* gene and one tumor showed insertion and deletion in the $(A)_8$ region in the *hMSH3* gene, and two of the three above tumors showed MSI in tri- and tetranucleotide repeats. One MSI-L tumor showed somatic alteration in a 9-bp repeat sequence in *hMSH3*. No frameshift mutations were found in the $(A)_7$ and $(A)_6$ regions in *hMSH3*. Thus, we confirmed that the $(A)_8$ region in *hMSH3* is a hot spot and mutations in the $(A)_7$ and $(A)_6$ regions in *hMSH3* are not common. The *hMSH3* mutation may enhance genomic instability in some colorectal cancers.

Key words: hMSH3 — Microsatellite instability (MSI) — Sporadic colon cancer — Mutations — PCR-SSCP

Homologs of bacterial DNA mismatch repair (MMR) proteins, MutS and MutL, have been purified in humans. A heterodimer hMutSα composed of MutS homologs, hMSH2 and hMSH6 (GTBP), recognizes single-base and one-base insertion/deletion mismatches, whereas hMutSβ composed of hMSH2 and hMSH3 recognizes 2–4 insertion/deletion mismatches. This mechanism has been shown to operate in an *in vitro* system,[1)] a *baculovirus* expression system,[2)] and human cells *in vivo*,[3–6)] although functional redundancy was observed between hMutSα and hMutSβ. Both heterodimers bind MutL homologs, hMLH1 and hPMS2, and each tetramer is thought to work as mismatch repair machinery.[7)] Mutant MutS homologs increase microsatellite instability in yeasts, which have similar molecular mechanisms of MMR to humans. The spectrum of the instability is different between the MSH6 mutant and the MSH3 mutant; the former characteristically shows single repeat alterations, while the latter shows prominent tract instability.[8, 9)]

Hereditary nonpolyposis colorectal cancer (HNPCC) is a hereditary cancer syndrome characterized by microsatellite instability (MSI). The genes responsible for HNPCC were cloned and identified as the MMR genes.[10–14)] Some sporadic cancers with MSI showed somatic mutations of the MMR genes.[15–17)] The mutated MMR genes in HNPCC and these sporadic cancers are mainly the *hMSH2* or *hMLH1* genes.[18)] Although germline mutations of *hMSH3* have not been reported in HNPCC, somatic mutations of *hMSH3* have been found in colorectal, stomach, pancreas, and endometrial carcinomas with MSI.[19, 20)] These mutations are deletions of an A residue in the $(A)_8$ region in exon 7 of the *hMSH3* gene. The same mutation was also found as a hot spot of somatic mutation in HNPCC patients.[21)] Mutations in the $(C)_8$ region in the *hMSH6* gene were also reported in colorectal and stomach carcinomas with MSI[19)] and as somatic mutations in HNPCC.[21)] Those reports suggest that mutations of *hMSH3* and *hMSH6* are not primary events in the tumorigenesis, and the $(A)_8$ region in the *hMSH3* gene and the $(C)_8$ region in the *hMSH6* gene are targets of MSI. In addition, the whole coding region of the *hMSH3* gene has not been analyzed; only the $(A)_8$ region was analyzed in those reports. Thus, mutation search over a wider region of the *hMSH3* gene is necessary to investigate the role of this gene in the tumorigenesis.

We cloned the *hMSH3* gene[22, 23)] and reported a polymorphism.[24)] In addition, we reported that the mutation of the *hMSH3* gene is associated with trinucleotide repeat instability in the coding region of the *E2F-4* gene.[25)] In

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this study, we analyzed MSI in polynucleotide repeat markers and the *hMSH3* gene in sporadic colon cancer. We found somatic mutations in the $(A)_8$ region and somatic change in the 9-bp repeat polymorphism in the *hMSH3* gene.

MATERIALS AND METHODS

Samples Genomic DNA of 79 Japanese sporadic colon cancer patients was extracted from surgically resected normal and cancer tissues of colon. These samples are the same as those that we used to examine transforming growth factor-β type II receptor (*TGF-β RII*) gene mutations.[26] The samples were extracted after informed consent had been obtained.

MSI MSI was screened by using 3 mono- and 4 dinucleotide repeat markers. For mononucleotide repeats, BAT25, BAT26, and the $(A)_{10}$ repeat of TGF-β RII were used, and for dinucleotide repeats, DCC, D5S107, D17S261, and the $(GT)_3$ repeat of TGF-β RII were used, as described elsewhere.[26] Then, the MSI samples were further analyzed using 3 tri- and 2 tetranucleotide repeat markers. For trinucleotide repeat, the CAG repeat of HD (the Huntington disease gene), SADNA2,[27] and E2F-4,[28] and for tetranucleotide repeat, D17S846[29] and F13A1[30] were employed. Polymerase chain reaction (PCR) was carried out with [γ-^{33}P]ATP-labeled forward primer and nonlabeled reverse primer. PCR products were applied to 6% polyacrylamide gel with 7 *M* urea and run at 60 W for 1–3 h. The gel was dried and exposed to an X-ray film at −70°C overnight.

Analysis of repeat number of A repeats in the *hMSH3* gene An $(A)_8$ repeat in exon 7, an $(A)_7$ repeat in exon 22, and $(A)_6$ repeats in exons 13, 15, and 19 in the *hMSH3* gene were analyzed in all 79 samples on 6% polyacrylamide gel electrophoresis with 7 *M* urea as described above with the primers used in PCR-SSCP (single strand conformation polymorphism) analysis.

Analysis of repeat number of C repeat in the *hMSH6* gene A $(C)_8$ repeat in exon 5 of the *hMSH6* gene was analyzed using the same procedures as for the A repeats of the *hMSH3* gene. PCR was performed as described,[19] and the MSI samples were analyzed.

PCR-SSCP analysis PCR-SSCP was performed in the MSI samples using primers that amplify 24 exons based on the genomic sequence of the *hMSH3* gene.[23] Whole exons except for exon 1 were amplified. In exon 1, the 3′-side of the exon, which is approximately two-thirds of the exon, was amplified. The sequences of the primers we used are available upon request. PCR was carried out with [γ-^{33}P]ATP-labeled primers. PCR conditions were 94°C for 5 min for the first denaturation, then 30 cycles with 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min followed by 72°C for 5 min for the final extension. For exon 1, 5% (final concentration) dimethyl sulfoxide was added. For exon 14, the annealing temperature was 50°C. Heat-denatured PCR products were applied to 0.5× MDE gel (FMC Bioproducts, Rockland, ME) and run at 6 W for 12 h at room temperature. The gel was dried and exposed to an X-ray film at −70°C for 3–5 days.

Direct nucleotide sequencing Each SSCP band was cut out and eluted with water, then the eluate was re-amplified with nonlabeled primers. The PCR product was labeled using an "ABI PRISM Dye Terminator Cycle Sequencing Kit" and nucleotide sequencing was carried out with an ABI 373 autosequencer (Perkin-Elmer Corporation, Norwalk, CT).

RESULTS

Three mono- and 4 dinucleotide repeat markers were screened in 79 samples and samples were classified into MSI-H and MSI-L according to the criteria decided in "The International Workshop on Microsatellite Instability and RER Phenotypes in Cancer Detection and Familial Predisposition."[31] Since BAT25 and BAT26 coincide with the markers in the reference panel of the criteria, but the other markers we used were not in this panel, although some markers were in alternative loci in the criteria, we calculated the percentage of positive markers. The tumors that showed MSI in ≥30–40% of markers were defined as MSI-H and tumors that showed MSI in <30–40% of markers were defined as MSI-L.[31] In this study, 5 samples (6%) were defined as MSI-H, 15 samples (19%) were MSI-L, and the rest (75%) were MSS (microsatellite stable) or MSI-L. There is no clear criterion to differentiate MSI-L and MSS, so we simply defined the samples that showed MSI in <30–40% of markers as MSI-L. We assessed additional microsatellite markers that contained 3 tri- and 2 tetranucleotide repeats and the $(C)_8$ repeat in the *hMSH6* gene in 17 MSI samples. The results on microsatellite markers are summarized in Table I. Three out of 5 MSI-H samples revealed alterations in these markers. The sample 11T that showed MSI in 6/7 (85%) of the markers revealed instability in HD and D17S846, and a hetero-insertion of a C residue in the $(C)_8$ repeat in *hMSH6*. The sample 26T that showed MSI in 5/7 (71%) of the markers revealed instability in D17S846 and insertion/deletion of an A residue in the $(A)_8$ repeat in *hMSH3* as well as a hetero-deletion of CAG in the $(CAG)_{14}$ repeat in *E2F-4*, which we reported previously.[25] The sample 22T that showed MSI in 4/7 (57%) of the markers revealed a hetero-insertion of C in the $(C)_8$ repeat in *hMSH6*. In summary, only one sample (26T) showed mutations in the *hMSH3* gene. No MSI-L samples showed instability in tri- and tetranucleotide repeat markers.

There are five adenine repeat sequences in the *hMSH3* gene. The $(A)_8$ repeat sequence in exon 7 has been reported as a hot spot of mutation in some cancers.[19, 20]

Table I. Microsatellite Alterations of the Markers and Mutations in the $(A)_8$ Region in the *hMSH3* Gene and the $(C)_8$ Region in the *hMSH6* Gene

Sample number	Mononucleotide[a)]	Dinucleotide[a)]	Trinucleotide			Tetranucleotide		*hMSH3* $(A)_8$	*hMSH6* $(C)_8$
			HD	SADNA2	E2F-4	D17S846	F13A1		
MSI-H									
11	3/3	3/4	+ (1+)	–	–	+ (1+)	–	–	+ (9/8)
22	3/3	1/4	–	–	–	–	–	–	+ (9/8)
26	3/3	2/4	–	–	+ (1–)[b)]	+ (1+)	–	+ (9/7)[b)]	–
79	2/3	2/4	–	–	–	–	–	–	–
100	3/3	2/4			ND			–	ND
MSI-L									
33	0/3	1/4	–	–	–	–	–	–	–
36	1/3	0/4	–	–	–	–	–	–	–
37	0/3	1/4	–	–	–	–	–	–	–
57	0/3	1/4			ND			–	ND
59	0/3	1/4	–	–	–	–	–	–	–
60	0/3	1/4	–	–	–	–	–	–	–
63	1/3	1/4	–	–	–	–	–	–	–
70	1/3	0/4	–	–	–	–	–	–	–
73	0/3	1/4	–	–	–	–	–	–	–
86	0/3	1/4	–	–	–	–	–	–	–
104	0/3	1/4				ND		–	ND
106	0/3	1/4	–	–	–	–	–	–	–
109	0/3	1/4	–	–	–	–	–	–	–
170	1/3	1/4	–	–	–	–	–	–	–
173	0/3	1/4	–	–	–	–	–	–	–

a) Previously reported.[26)]
b) The results of E2F-4 and 7 repeats in the $(A)_8$ region were previously reported.[25)]
In the tri- and tetranucleotide markers, numbers in parentheses show somatic change of repeat number; 1+ indicates an increase of one repeat compared with the normal tissue.
In the *hMSH3* and *hMSH6* genes, numbers in parentheses indicate the repeat number of both alleles.
ND: not determined.

The $(A)_7$ repeat in exon 22 and $(A)_6$ repeats in exons 13, 15, and 19 have not been studied. We searched for mutation in these adenine repeat sequences in 79 colon cancer samples by electrophoresis with urea gel and SSCP analysis, but we did not find any frameshift mutations in exons 13, 15, 19, and 22. Although we had already reported a deletion of A in the $(A)_8$ region of exon 7 in the sample 26T,[25)] we found also an insertion of A in the same region (Fig. 1). This occurred only in the tumorous tissue, suggesting that the frameshift was somatic. These mutations cause premature termination of the encoded protein.

We had reported length polymorphisms of the 9-bp repeat sequence in exon 1 of the *hMSH3* gene, which corresponds to cDNA residues 151–204.[24)] In this study, a somatic alteration of the repeat number was found in the sample 63T from 6 to 7 repeats compared with 63N (Fig. 2). This alteration occurred in the MSI-L sample, and would not change the reading frame, but was apparently cancer-specific.

DISCUSSION

MMR genes have been shown to be responsible for HNPCC and sporadic cancers with MSI, although the *hMSH3* gene has not been proved a responsible gene for HNPCC. We assayed MSI in 12 markers that contain 3 mononucleotide, 4 dinucleotide, 3 trinucleotide, and 2 tetranucleotide repeats. Since hMSH3 binds to hMSH2 to constitute hMutSβ, which recognizes mainly 2–4 loop mismatches, alteration of hMSH3 function may influence instability in polynucleotide repeats, whereas defective hMSH6 function may be associated with mononucleotide instability. Previously, we reported that the *hMSH3* gene mutation was closely related to *E2F-4* gene instability, but was not related to other trinucleotide markers.[25)] The instability of the CAG repeat in the *E2F-4* gene revealed in this study was a hetero-deletion in the tumor 26T, which leads to a shorter polyserine domain that may be essential for transactivation, and may function in a dominant positive manner.[25)]

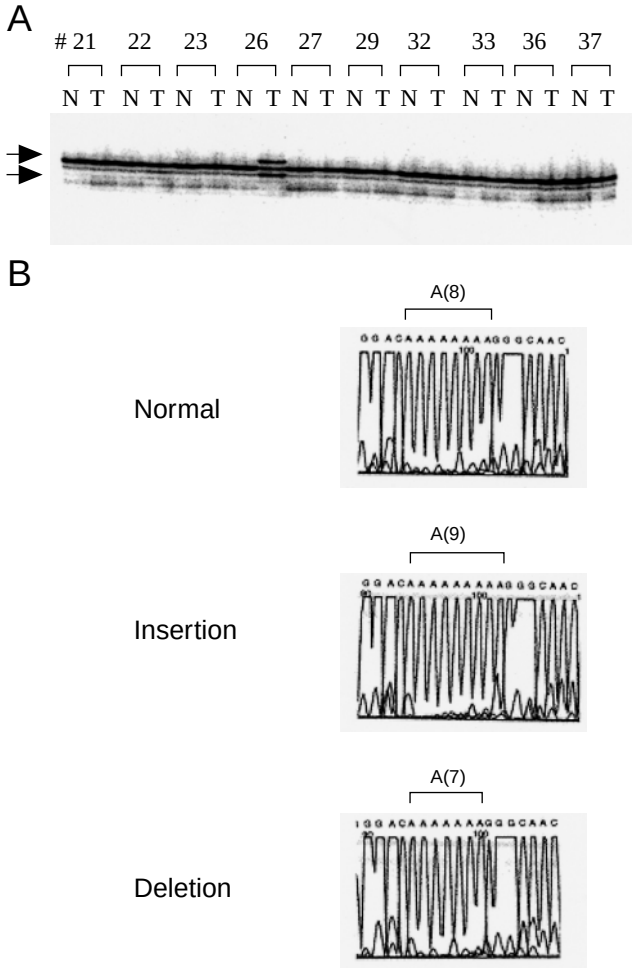

Fig. 1. Frameshift mutations in exon 7 of the *hMSH3* gene. A shows an autoradiogram of 6% polyacrylamide gel electrophoresis with 7 *M* urea. The sample 26T revealed both insertion and deletion of A residues (arrows). B shows nucleotide sequence patterns of both alleles in the sample 26T.

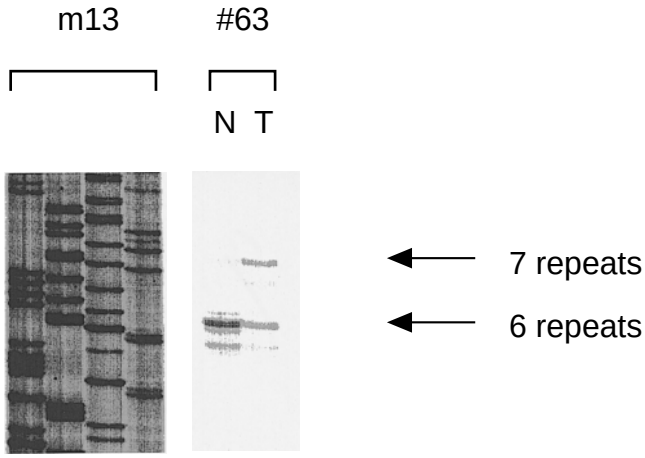

Fig. 2. Length polymorphism in exon 1 of the *hMSH3* gene. An autoradiogram of 6% polyacrylamide gel electrophoresis with 7 *M* urea is shown. The sample 63T showed 6 and 7 repeats, while 63N showed only 6 repeats. "m13" indicates the nucleotide sequence ladder of m13 phage DNA as a nucleotide size standard.

We searched almost the whole coding region of the *hMSH3* gene in 19 MSI samples and 5 adenine repeat sequences in 79 samples, and found both insertion and deletion of an A residue in the same cancer sample, 26T. There are reports of deletion of A, but to our knowledge, insertion has never been reported. The deletion and the insertion in the sample 26T seemed to occur on both alleles, but alternatively, and the cancer tissue may have contained two different clones. These mutations were somatic and cancer-specific because normal tissue from the same patient revealed no mutation in the *hMSH3* gene. Both mutant alleles would cause premature termination of the encoded protein. Therefore, if the mutations occurred on both alleles, the truncated proteins would lose the physiological function of hMSH3. This biallelic inactivation may contribute to genomic instability in the tumor 26T. Since the frequency with which one cancer has homozygous somatic mutations should be low, the $(A)_8$ region seems to be the predominant target of MSI.

Malkhosyan *et al.*[19] reported frameshift mutations in the $(A)_8$ region in the *hMSH3* gene and in the $(C)_8$ region in the *hMSH6* gene. The incidence of mutation in colorectal cancer with MSI was 39% for the $(A)_8$ and 30% for the $(C)_8$. In this study, we found mutation in the $(A)_8$ region in the *hMSH3* gene in one patient (20%) and in the $(C)_8$ region in the *hMSH6* gene in two patients (40%) among 5 MSI-H sporadic colon cancer patients. In HNPCC with MSI, the incidence of somatic mutations in the $(A)_8$ region in the *hMSH3* gene was reported as 58%, while that in the $(C)_8$ region in the *hMSH6* gene was 26%.[21] In addition, we did not find any frameshift mutations in the $(A)_7$ and $(A)_6$ regions in the *hMSH3* gene, suggesting that the $(A)_8$ region in exon 7 is a hot spot and mutations in the other A repeats are not common.

We found another cancer-specific alteration in a 9-bp repeat sequence in the sample 63T. In this alteration, the repeat number was increased, but the reading frame was unchanged. Although the sample 63T was classified into MSI-L, it showed MSI in two out of 12 loci. We had reported length polymorphism of this 9-bp repeat sequence,[24] in which both 6 and 7 repeats were found in the normal population. Those findings indicate that this alteration of the repeat number may not change the protein

function. However, this alteration may be one of the targets of genomic instability.

The present study suggests that genomic instability, which results from defective mismatch repair function, may target specific repetitive sequences, namely the $(A)_8$ region in exon 7 and the 9-bp repeat sequence in exon 1 of the *hMSH3* gene. Although we could not assess the differentiation level of the tumors and mutations of the *hMSH2* and *hMLH1* genes, which are the major MMR genes associated with MSI, high frequency of MSI in the MSI-H tumors suggested that MMR function is likely to be defective in these tumors. The frameshift mutations in the $(A)_8$ region, which is a hot spot of *hMSH3* mutation, cause a truncated hMSH3 protein. The resultant mutant proteins make mismatch repair less effective, which in turn, enhances genomic instability, especially polynucleotide repeat instability as represented by E2F-4 and D17S846. This acceleration of the instability caused by "mutator mutations" of the *hMSH3* gene as well as the *hMSH6* gene, may be one of the important mechanisms of tumorigenesis in cancers with microsatellite instability.

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REFERENCES

1) Acharya, S., Wilson, T., Gradia, S., Kane, M. F., Guerrette, S., Marsischky, G. T., Kolodner, R. and Fishel, R. hMSH2 forms specific mispair-binding complexes with hMSH3 and hMSH6. *Proc. Natl. Acad. Sci. USA*, **93**, 13629–13634 (1996).
2) Palombo, F., Iaccarino, I., Nakajima, E., Ikejima, M., Shimada, T. and Jiricny, J. hMutSβ, a heterodimer of hMSH2 and hMSH3, binds to insertion/deletion loops in DNA. *Curr. Biol.*, **6**, 1181–1184 (1996).
3) Drummond, J. T., Genschel, J., Wolf, E. and Modrich, P. *DHFR/MSH3* amplification in methotrexate-resistant cells alters the hMutSα/hMutSβ ratio and reduces the efficiency of base-base mismatch repair. *Proc. Natl. Acad. Sci. USA*, **94**, 10144–10149 (1997).
4) Marra, G., Iaccarino, I., Lettieri, T., Roscilli, G., Delmastro, P. and Jiricny, J. Mismatch repair deficiency associated with overexpression of the *MSH3* gene. *Proc. Natl. Acad. Sci. USA*, **95**, 8568–8573 (1998).
5) Genschel, J., Littman, S. J., Drummond, J. T. and Modrich, P. Isolation of MutSβ from human cells and comparison of the mismatch repair specificities of MutSβ and MutSα. *J. Biol. Chem.*, **273**, 19895–19901 (1998).
6) Nakajima, E., Ikejima, M., Watanabe, A. and Shimada, T. Identification of the protein components of mismatch binding complexes in human cells using a gel-shift assay. *FEBS Lett.*, **453**, 85–89 (1999).
7) Modrich, P. and Lahue, R. Mismatch repair in replication fidelity, genetic recombination, and cancer biology. *Annu. Rev. Biochem.*, **65**, 101–133 (1996).
8) Strand, M., Earley, M. C., Crouse, G. F. and Petes, T. D. Mutations in the *MSH3* gene preferentially lead to deletions within tracts of simple repetitive DNA in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA*, **92**, 10418–10421 (1995).
9) Johnson, R. E., Kovvali, G. K., Prakash, L. and Prakash, S. Requirement of the yeast *MSH3* and *MSH6* genes for *MSH2*-dependent genomic stability. *J. Biol. Chem.*, **271**, 7285–7288 (1996).
10) Fishel, R., Lescoe, M. K., Rao, M. R. S., Copeland, N. G., Jenkins, N. A., Garber, J., Kane, M. and Kolodner, R. The human mutator gene homolog *MSH2* and its association with hereditary nonpolyposis colon cancer. *Cell*, **75**, 1027–1038 (1993).
11) Leach, F. S., Nicolaides, N. C., Papadopoulos, N., Liu, B, Jen, J., Parsons, R., Peltomäki, P., Sistonen, P., Aaltonen, L. A., Nyström-Lahti, M., Guan, X.-Y., Zhang, J., Meltzer, P. S., Yu, J.-W., Kao, F.-T., Chen, D. J., Cerosaletti, K. M., Fournier, R. E. K., Todd, S., Lewis, T., Leach, R. J., Naylor, S. L., Weissenbach, J., Mecklin, J.-P., Järvinen, H., Petersen, G. M., Hamilton, S. R., Green, J., Jass, J., Watson, P., Lynch, H. T., Trent, J. M., de la Chapelle, A., Kinzler, K. W. and Vogelstein, B. Mutations of a *mutS* homolog in hereditary nonpolyposis colorectal cancer. *Cell*, **75**, 1215–1225 (1993).
12) Papadopoulos, N., Nicolaides, N. C., Wei, Y.-F., Ruben, S. M., Carter, K. C., Rosen, C. A., Haseltine, W. A., Fleischmann, R. D., Fraser, C. M., Adams, M. D., Venter, J. C., Hamilton, S. R., Petersen, G. M., Watson, P., Lynch, H. T., Peltomäki, P., Mecklin, J.-P., de la Chapelle, A., Kinzler, K. W. and Vogelstein, B. Mutation of a *mutL* homolog in hereditary colon cancer. *Science*, **263**, 1625–1629 (1994).
13) Bronner, C. E., Baker, S. M., Morrison, P. T., Warren, G., Smith, L. G., Lescoe, M. K., Kane, M., Earabino, C., Lipford, J., Lindblom, A., Tannergård, P., Bollag, R. J., Godwin, A. R., Ward, D. C., Nordenskjøld, M., Fishel, R., Kolodner, R. and Liskay, R. M. Mutation in the DNA mismatch repair gene homologue *hMLH1* is associated with hereditary non-polyposis colon cancer. *Nature*, **368**, 258–261 (1994).
14) Nicolaides, N. C., Papadopoulos, N., Liu, B., Wei, Y.-F., Carter, K. C., Ruben, S. M., Rosen, C. A., Haseltine, W. A.,

Fleischmann, R. D., Fraser, C. M., Adams, M. D., Venter, J. C., Dunlop, M. G., Hamilton, S. R., Petersen, G. M., de la Chapelle, A., Vogelstein, B. and Kinzler, K. W. Mutations of two *PMS* homologues in hereditary nonpolyposis colon cancer. *Nature*, **371**, 75–80 (1994).

15) Liu, B., Nicolaides, N. C., Markowitz, S., Willson, J. K. V., Parsons, R. E., Jen, J., Papadopoulos, N., Peltomäki, P., de la Chapelle, A., Hamilton, S. R., Kinzler, K. W. and Vogelstein, B. Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. *Nat. Genet.*, **9**, 48–55 (1995).
16) Børrensen, A.-L., Lothe, R. A., Meling, G. I., Lystad, S., Morrison, P., Lipford, J., Kane, M. F., Rognum, T. O. and Kolodner, R. D. Somatic mutations in the *hMSH2* gene in microsatellite unstable colorectal carcinomas. *Hum. Mol. Genet.*, **4**, 2065–2072 (1995).
17) Moslein, G., Tester, D. J., Lindor, N. M., Honchel, R., Cunningham, J. M., French, A. J., Halling, K. C., Schwab, M., Goretzki, P. and Thibodeau, S. N. Microsatellite instability and mutation analysis of *hMSH2* and *hMLH1* in patients with sporadic, familial and hereditary colorectal cancer. *Hum. Mol. Genet.*, **5**, 1245–1252 (1996).
18) Eshleman, J. R. and Markowitz, S. D. Mismatch repair defects in human carcinogenesis. *Hum. Mol. Genet.*, **5**, 1489–1494 (1996).
19) Malkhosyan, S., Rampino, N., Yamamoto, H. and Perucho, M. Frameshift mutator mutations. *Nature*, **382**, 499–500 (1996).
20) Risinger, J. I., Umar, A., Boyd, J., Berchuck, A., Kunkel, T. A. and Barrett, J. C. Mutation of *MSH3* in endometrial cancer and evidence for its functional role in heteroduplex repair. *Nat. Genet.*, **14**, 102–105 (1996).
21) Akiyama, Y., Tsubouchi, N. and Yuasa, Y. Frequent somatic mutations of *hMSH3* with reference to microsatellite instability in hereditary nonpolyposis colorectal cancers. *Biochem. Biophys. Res. Commun.*, **236**, 248–252 (1997).
22) Fujii, H and Shimada, T. Isolation and characterization of cDNA clones derived from the divergently transcribed gene in the region upstream from the human dihydrofolate reductase gene. *J. Biol. Chem.*, **264**, 10057–10064 (1989).
23) Watanabe, A., Ikejima, M., Suzuki, N. and Shimada, T. Genomic organization and expression of the human MSH3 gene. *Genomics*, **31**, 311–318 (1996).
24) Nakajima, E., Orimo, H., Ikejima, M. and Shimada, T. Nine-bp repeat polymorphism in exon 1 of the *hMSH3* gene. *Jpn. J. Hum. Genet.*, **40**, 343–345 (1995).
25) Ikeda, M., Orimo, H., Moriyama, H., Nakajima, E., Matsubara, N., Mibu, R., Tanaka, N., Shimada, T., Kimura, A. and Shimizu, K. Close correlation between mutations of *E2F4* and *hMSH3* genes in colorectal cancers with microsatellite instability. *Cancer Res.*, **58**, 594–598 (1998).
26) Orimo, H., Ikejima, M., Nakajima, E., Emi, M. and Shimada, T. A novel missense mutation and frameshift mutations in the type II receptor of transforming growth factor-β gene in sporadic colon cancer with microsatellite instability. *Mutat. Res.*, **382**, 115–120 (1998).
27) Goellner, G. M., Tester, D., Thibodeau, S., Almqvist, E., Goldberg, Y. P., Hayden, M. R. and McMurray, C. T. Different mechanisms underlie DNA instability in Huntington disease and colorectal cancer. *Am. J. Hum. Genet.*, **60**, 879–890 (1997).
28) Yoshitaka, T., Matsubara, N., Ikeda, M., Tanino, M., Hanafusa, H., Tanaka, N. and Shimizu, K. Mutations of E2F-4 trinucleotide repeats in colorectal cancer with microsatellite instability. *Biochem. Biophys. Res. Commun.*, **227**, 553–557 (1996).
29) Flejter, W. L., Kukowska-Latallo, J. F., Kiousis, S., Chandrasekharappa, S. C., King, S. E. and Chamberlain, J. S. Tetranucleotide repeat polymorphism at D17S846 maps within 40 kb of GAS at 17q12-q22. *Hum. Mol. Genet.*, **2**, 1080 (1993).
30) Polymeropoulos, M. H., Rath, D. S., Xiao, H. and Merril, C. R. Tetranucleotide repeat polymorphism at the human coagulation factor XIII a subunit gene (F13A1). *Nucleic Acids Res.*, **19**, 4306 (1991).
31) Boland, C. R., Thibodeau, S. N., Hamilton, S. R., Sidransky, D., Eshleman, J. R., Burt, R. W., Meltzer, S. J., Rodriguez-Bigas, M. A., Fodde, R., Ranzani, G. N. and Srivastava, S. A National Cancer Institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res.*, **58**, 5248–5257 (1998).