Analysis of a correlation between the *BRAF* V600E mutation and abnormal DNA mismatch repair in patients with sporadic endometrial cancer

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Abstract. Point mutations of *KRAS* and *BRAF* genes are thought to be important in carcinogenesis of colon cancer. In particular, gene instability caused by decreased expression of the *hMLH1* gene, a DNA mismatch repair (MMR) gene, may be linked to the activating *BRAF* V600E point mutation in sporadic colon cancer. However, a consensus has not been established regarding the correlation between point mutations of *KRAS* or *BRAF* and carcinogenesis in patients with endometrial cancer, which is closely related to colon cancer. Therefore, we analyzed aberrant hypermethylation of the *hMLH1* gene, microsatellite instability (MSI), and point mutations of *KRAS* and *BRAF* in 44 samples of sporadic endometrial cancer, with the aim of examining the mechanism of carcinogenesis in patients with endometrial cancer. Aberrant *hMLH1* hypermethylation was found in 17 of the 44 cases (38.6%) and showed a significant positive correlation with MSI (p=0.02). This suggests that an abnormal MMR mechanism plays an important role in carcinogenesis of sporadic endometrial cancer. Point mutation of *KRAS* was found in 6 of the 44 cases (13.6%), but no *BRAF* V600E mutation was detected. These data suggest that the *BRAF* V600E mutation is not the target gene for abnormal MMR in carcinogenesis in patients with sporadic endometrial cancer, unlike in colon cancer. This is supported by the relatively few previous reports indicating a correlation between endometrial cancer and the *BRAF* V600E mutation. Identification of new candidates for the target gene for abnormal MMR in endometrial cancer requires further work.

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Introduction

A cancer may develop as a result of repeated mutation of genes involved in differentiation or proliferation. Such a multi-step mechanism of carcinogenesis with mutation of multiple cancer-related genes is often observed in patients with colon cancer. The correlation between colon cancer carcinogenesis and point mutation of *RAS*/*RAF* genes in the *MAP* kinase pathway suggests that these genes have an important role at an early stage of malignant alteration of colon cancer (1).

Endometrial cancer has many similarities with colon cancer and is detected at high rates as a double cancer of hereditary non-polyposis colon cancer (HNPCC). Germline mutation of *hMLH1*, a DNA mismatch repair (MMR) gene, occurs at high rates in HNPCC patients (2), and decreased expression of *hMLH1* due to aberrant hypermethylation has also been found in patients with sporadic colon cancer and endometrial cancer (3). Decreased expression of *hMLH1* due to epigenetic changes may facilitate gene replication errors and cause gene instability, which can be detected as microsatellite instability (MSI) (4). Microsatellite DNA is a region with short repeated sequences of 1-2 bases, and PCR-based detection of replication errors in this region has been used widely as a clinical test to examine gene instability. Such instability may cause mutation of cancer-related genes, and a correlation between MSI due to decreased *hMLH1* expression and point mutations of *KRAS* and *BRAF* genes has been proposed in patients with colon cancer (5,6).

Mutation of the *BRAF* gene has been found in many human cancers, including colon cancer, malignant melanomas, thyroid carcinoma and ovarian carcinoma (7-9). *BRAF* is one of the 3 subtypes of *RAF* family genes and encodes a tyrosine kinase involved in mitogenic signaling in the *RAS-RAF-MEK-ERK-MAP* kinase pathway. The function of *RAF* is regulated by *RAS*, and an activating point mutation of *BRAF* causes unregulated constitutive activation of the tyrosine kinase activity and facilitates cell proliferation via the *MAP* kinase pathway. The V600E mutation in exon 15 of *BRAF* is of particular interest, since tyrosine kinase activity 10-fold that of wild-type has been found in tumor tissue with this mutation (10). The V600E mutation is found in about 15% of patients with sporadic colon cancer and can be used for clinical diagnosis of non-inheritive sporadic colon cancer (10). Furthermore, since *BRAF* V600E is observed in 32% of cases of MSI-positive

Table I. Primer sequences used in PCR and MSP analysis.

Gene	PCR method	Sense	Antisense	Size (bp)	Annealing (˚C)
hMLH1	Methylated	ACGTAGACGTTTTATTAGGGTCGC	CCTCATCGTAACTACCCGCG	112	60
	Unmethylated	TTTTGATGTAGATGTTTTATTAGGGTTGT	ACCACCTCATCATAACTACCCACA	124	60
KRAS	Codons 12, 13	GCCTGCTGAAAATGACTGAAT	TTATCTGTATCAAAGAATGGTC	180	64
BRAF	Codon 600	TCATAATGCTTGCTCTGATAGGA	GGCCAAAAATTTAATCAGTGGA	150	60

sporadic colon cancer and 75% of cases with sporadic colon cancer with aberrant hypermethylation of *hMLH1*, *BRAF* has been proposed as the target gene of abnormal MMR (11).

In contrast to colon cancer, only a few reports have shown mutation of *BRAF* in patients with endometrial cancer. Feng *et al* found *BRAF* mutations in 21% of patients with endometrial cancer and suggested that the mutation correlated with decreased *hMLH1* expression (12). However, Salvesen *et al* found a *BRAF* mutation in only 2% of patients with endometrial cancer (13). Therefore, it is unclear whether mutation of *BRAF* is important in carcinogenesis of endometrial cancer and whether the mutation may be linked to abnormal expression of the *hMLH1* gene. In this study, we analyzed aberrant hypermethylation of *hMLH1,* MSI, and mutations of *KRAS* and *BRAF* in patients with sporadic endometrial cancer to examine correlations among point mutations in *RAS/RAF* family genes, abnormal MMR caused by aberrant *hMLH1* hypermethylation, and carcinogenesis of sporadic endometrial cancer.

Materials and methods

Cell lines. Eight cell strains were used in the study: HEC108, Ishikawa (a human endometrial cancer-derived cultured cell line supplied by Dr Hiroyuki Kuramoto); HOOUA and HHUA (supplied by Dr Isamu Ishiwata); and SNG-II, SNG-M, HEC-1B and KLE. KLE cells were cultured in a DMEM/F12 (1:1) medium (Gibco-BRL, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS) (Sanko Junyaku Co., Tokyo, Japan). All other cells were cultured in 10% FBS-supplemented F12 medium (Sigma, St. Louis, MO, USA). The cells were incubated in a dish of 10 cm in diameter under 5% CO_2 at 37˚C.

Clinical specimens. The subjects were 44 patients with endometrial cancer (G1, 20; G2, 11; G3, 13) who gave informed consent to collection of cancer specimens. Of these patients, 37 had endometrioid adenocarcinoma and 7 had adenosquamous carcinoma. The grade of histological differentiation (G1-G3) and the cancer stage at surgery were determined based on the Guidelines for Endometrial Cancer published by the Japan Society of Obstetrics and Gynecology.

DNA extraction and methylation-specific PCR (MSP) in the hMLH1 promoter region. DNA was extracted from the 44 endometrial cancer specimens using liquid-based cytology with a Get Pure DNA Kit (Dojindo Molecular Technologies, Inc., Kumamoto, Japan). Distilled water was added to 1 μg of the extracted DNA up to a volume of 50 μl and 5.5 μl of 3 N NaOH solution was added. After mixing, the solution was incubated at 37˚C for 15 min, and then 520 μl of 3 M sodium bisulfite (Sigma) prepared at pH 5.5 with 30 μl of 10 mM hydroquinone (Sigma) and 10 N NaOH was added to the solution. After mixing in an upturned position to prevent vaporization, the solution was overlaid with mineral oil and incubated at 50˚C overnight. Next, 1 ml of clean-up resin (Promega, Madison, WI, USA) was added to the lower layer and the resulting solution was mixed in an upturned position and then injected into a column. The column was rinsed with 2 ml of 80% isopropanol and then centrifuged at 15,000 rpm for 3 min to remove the isopropanol completely. Next, 50 μl of distilled water (70˚C) was added directly to the column, which was then centrifuged at 15,000 rpm for 2 min to extract DNA adsorbed on the column. Then, 5.5 μl of 2 N NaOH was added to the resulting DNA solution. After mixing, the solution was incubated at 37˚C for 20 min, after which 66 μl of 5 N ammonium acetate solution and 243 μl of 95% ethanol were added and the solution was incubated at 80˚C for 1 h and centrifuged at 15,000 rpm for 30 min to precipitate DNA. Approximately 50 μl of the supernatant was left in the tube. The rest of the supernatant was collected, mixed with 1 ml of 70% ethanol, and then centrifuged at 15,000 rpm for 30 min to rinse the DNA. The precipitated DNA was air-dried and dissolved in 20 μl of distilled water; 2 μl of this solution was used as the MSP template solution. AmpliTaq Gold and 10X PCR buffer/$MgCl_2$ with dNTP (Applied Biosystems, Foster City, CA, USA) was used in PCR analysis and DNA was analyzed using a GeneAmp PCR System 9700 (Applied Biosystems). The PCR primer sequences are shown in Table I. DNA extracted from the cultured cell lines was also used in MSP analysis of *hMLH1* (14).

Microsatellite instability analysis. Genomic DNA extracted from normal and tumor tissue samples from the 44 patients with endometrial cancer was PCR amplified at the microsatellite repeat loci D2S123, D5S346, D17S250, BAT26, BAT25, MSH3, MSH6, TGF-ßRII, BAX, MBD4A10 and MBD4A6, which include 3 dinucleotide (CA) and 8 mononucleotide repeats as microsatellite markers. PCR reactions were performed in a total volume of 25 μl containing 10X buffer, 0.125 mM deoxynucleoside triphosphate, 0.2 μM of each primer, and 0.25 units of TaqDNA polymerase. The PCR

conditions were as follows: 94˚C for 10 min; 30 cycles at 94˚C for 45 sec, 58˚C for 45 sec, and 72˚C for 40 sec; followed by a final extension step at 72˚C for 10 min. After PCR, 1 μl of the product was mixed with 12 μl of loading buffer containing formamide and Rox size standards. This mixture was denatured at 95˚C for 2 min and cooled on ice before loading onto an ABI PRISM 310 sequencer (Applied Biosystems). The results were analyzed using GeneScan software (Applied Biosystems). Tumors were classified as MSI-H when ≥30% of these markers showed MSI, in accordance with the recent recommendation of the National Cancer Institute Workshop. Low-frequency MSI (<30% of 11 markers) was included in the category of MSI-L and alteration of even one microsatellite region led to definition of the patient as MSI-positive (15).

Determination of KRAS and BRAF mutations. DNA was extracted from the 8 endometrial cancer-derived cell lines and 44 endometrial cancer specimens using liquid-based cytology with a Get Pure DNA Kit (Dojindo Molecular Technologies). Individual point mutations of the *KRAS* and *BRAF* genes were documented using two gene-specific oligonucleotide primer pairs designed for PCR amplification of the region of the *KRAS* gene harboring codons 12 and 13 and the region of exon 15 of the *BRAF* gene encompassing codon 600, respectively. The oligonucleotide primers for sequencing of *KRAS* and *BRAF* are shown in Table I. Each exon was amplified by PCR using 0.5 Ag of template DNA, sense and antisense primers, and an AmpliTaq Gold PCR kit (Applied Biosystems). A total of 50 μl of reaction mixture was prepared according to the manufacturer's instructions and PCR was commenced at 94˚C for 3 min; followed by 35 cycles of 94˚C for 30 sec, 64˚C or 60˚C for 30 sec, and 72˚C for 1 min; with a final extension step for 5 min. The PCR products were purified using an UltraClean PCR Clean-up kit (Mobio Laboratories, Solana Beach, CA) and subjected to direct sequencing using purified products and the same sets of primers in a capillary automatic sequencer (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems). Sequence data were analyzed using the Basic Local Alignment Search Tool (BLAST) software located at the National Center for Biotechnology Information web site (http://www.ncbi.nlm.nih.gov) (12).

Statistical analysis. Correlation of *KRAS* mutations with the grade of histological differentiation and the cancer stage at surgery were analyzed using the χ^2 test and Mann-Whitney test, respectively. Correlation of *KRAS* mutations with patient age was also examined, after establishing that age had a normal distribution in the groups of patients with and without *KRAS* mutations. Mann-Whitney test was used to examine whether the population medians of the two independent groups differed significantly. Correlation of aberrant DNA hypermethylation of *hMLH1* with MSI was analyzed by the χ^2 test.

Results

MSP analysis of samples of endometrial cancer showed aberrant *hMLH1* hypermethylation in 17 of the 44 cases (38.6%) (Fig. 1, Table II). In MSI analysis, 31.8% (14 samples), 6.8% (3 samples), and 61.4% (27 samples) of the cases were categorized as MSI-H, MSI-L and MSS (microsatellite stability), respectively; that is, 38.6% were judged to be MSI-positive. Aberrant hypermethylation of the *hMLH1* gene was found in a higher number of MSI-positive cases, with a statistically significant positive correlation (p=0.02) between abnormal *hMLH1* methylation and MSI (Table III).

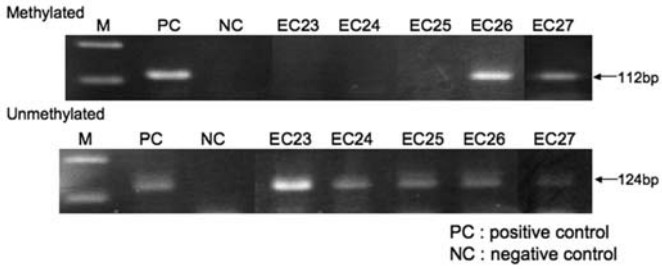

Figure 1. Detection of abnormal hypermethylation of the *hMLH1* gene in endometrial cancer using MSP analysis. The 112 bp band indicating abnormal hypermethylation was found in EC26 and EC27.

A point mutation at codon 12 of *KRAS* was found in 3 (HEC-1B, HHUA and SNG-M) of the 8 endometrial cancer-derived cell lines that were examined. These changes resulted in a G-D mutation in one cell line and G-V mutations in the other 2 cell lines. None of the cell lines had a point mutation at codon 13 of *KRAS* or at codon 600 of *BRAF* (Table IV). A point mutation at codon 12 of *KRAS* was observed in 6 of the 44 samples of endometrial cancer (13.6%) (Fig. 2, Table II), with a similar mutation to those in the cultured cell lines (G-D or V) in 5 of the 6 cases (83.3%). The point mutation at codon 12 of *KRAS* showed no correlation with clinicopathological characteristics of endometrial cancer or with age upon development of cancer, but tended to occur more frequently in well-differentiated adenocarcinoma (p=0.1, Table V). There were no correlations among aberrant *hMLH1* hypermethylation, MSI, and point mutation at codon 12 of *KRAS*. No point mutation at codon 13 of *KRAS* (Table II) or at codon 600 of *BRAF* (Table VI) was found in the 44 clinical samples of endometrial cancer.

Discussion

Carcinogenesis of colon cancer has been correlated with point mutation of the *RAS*/*RAF* family of genes in the MAP kinase pathway, suggesting the importance of mutation of these genes in an early stage of malignant change in colon cancer (1). Since mutations of *KRAS* and *BRAF* are observed in many MSI-positive cases of sporadic colon cancer with aberrant hypermethylation of the *hMLH1* gene, a correlation with MSI caused by decreased expression of hypermethylated *hMLH1* has been suggested (5). Similar decreased expression of *hMLH1* due to aberrant hypermethylation has been reported in endometrial cancer (14), but the correlation with point mutations of *KRAS* and *BRAF* remains unclear.

In the present study, aberrant hypermethylation of *hMLH1* was found in 38.7% of cases of sporadic endometrial cancer. Expression of *hMLH1* is significantly reduced by aberrant hypermethylation (14) and this may induce gene instability that can be detected as microsatellite instability (MSI). Previous studies have shown that about 13% of cases of sporadic colon cancer are MSI-positive (16) and that 84% of cases of MSI-

Table II. Results of MSI analysis, MSP analysis, and analysis of *BRAF* and *KRAS* gene mutations in cases of endometrial cancer.

No.	Age	Type	Stage	Grade	MSI	*hMLH1*	*BRAF* mutation Codon 600 GTG(V)	*KRAS* mutation Codon 12 GGT(G)	*KRAS* mutation Codon 13 GGC(G)
EC1	52	EM	Ib	G3	MSI-H	M	GTG	GGT	GGC
EC2	51	EM	IIIc	G1	MSI-H	U	GTG	GGT	GGC
EC3	54	AS	IIIc	G3	MSI-H	M	GTG	GGT	GGC
EC4	53	EM	Ib	G3	MSI-H	U	GTG	GGT	GGC
EC5	69	EM	IIIc	G2	MSI-H	M	GTG	GGT	GGC
EC6	55	EM	IIIc	G2	MSI-H	M	GTG	GGT	GGC
EC7	54	EM	Ia	G1	MSI-H	U	GTG	GGT	GGC
EC8	63	EM	Ia	G1	MSI-H	M	GTG	GGT	GGC
EC9	58	EM	Ib	G2	MSI-H	M	GTG	GGT	GGC
EC10	50	EM	IIIa	G3	MSI-H	U	GTG	GGT	GGC
EC11	61	EM	Ib	G1	MSI-H	M	GTG	GGT	GGC
EC12	55	AS	IVb	G2	MSI-H	U	GTG	GGT	GGC
EC13	78	EM	Ib	G3	MSI-H	U	GTG	GGT	GGC
EC14	65	EM	Ib	G2	MSI-H	M	GTG	GGT	GGC
EC15	61	EM	IIb	G1	MSI-L	U	GTG	GGT	GGC
EC16	57	EM	Ib	G3	MSI-L	U	GTG	GGT	GGC
EC17	41	EM	Ib	G1	MSI-L	M	GTG	GGT	GGC
EC18	50	EM	Ia	G1	MSS	U	GTG	GGT	GGC
EC19	61	EM	Ib	G1	MSS	M	GTG	GAT(D)	GGC
EC20	70	EM	IIIc	G2	MSS	U	GTG	GGT	GGC
EC21	62	AS	IIIa	G2	MSS	U	GTG	GCT(A)	GGC
EC22	40	EM	IIa	G1	MSS	U	GTG	GGT	GGC
EC23	59	EM	IIa	G3	MSS	U	GTG	GGT	GGC
EC24	80	EM	IIIc	G3	MSS	U	GTG	GGT	GGC
EC25	54	AS	Ib	G1	MSS	U	GTG	GGT	GGC
EC26	42	EM	IIb	G1	MSS	M	GTG	GGT	GGC
EC27	71	EM	IIIc	G3	MSS	M	GTG	GGT	GGC
EC28	60	EM	Ib	G1	MSS	U	GTG	GGT	GGC
EC29	57	EM	IIIa	G2	MSS	U	GTG	GGT	GGC
EC30	71	EM	IIa	G1	MSS	U	GTG	GTT(V)	GGC
EC31	37	EM	IIa	G2	MSS	M	GTG	GGT	GGC
EC32	47	EM	IIIb	G1	MSS	M	GTG	GAT(D)	GGC
EC33	67	EM	Ic	G2	MSS	U	GTG	GGT	GGC
EC34	53	EM	Ia	G1	MSS	M	GTG	GGT	GGC
EC35	62	AS	Ib	G1	MSS	M	GTG	GGT	GGC
EC36	56	EM	IIIc	G3	MSS	U	GTG	GGT	GGC
EC37	71	EM	Ib	G2	MSS	U	GTG	GAT(D)	GGC
EC38	53	EM	Ib	G3	MSS	U	GTG	GGT	GGC
EC39	42	AS	IIIc	G3	MSS	U	GTG	GGT	GGC
EC40	55	EM	Ic	G3	MSS	U	GTG	GGT	GGC
EC41	34	AS	IIIc	G1	MSS	U	GTG	GTT(V)	GGC
EC42	61	EM	Ic	G1	MSS	U	GTG	GGT	GGC
EC43	61	EM	Ic	G1	MSS	U	GTG	GGT	GGC
EC44	59	EM	Ib	G1	MSS	U	GTG	GGT	GGC

Table III. Correlation between MSI and abnormal hypermethylation of the *hMLH1* gene in cases of endometrial cancer.

	hMLH1		
	M	U	
MSI	10	7	
MSS	7	20	p=0.02

MSI, microsatellite instability; MSS, microsatellite stability; M, methylated; U, unmethylated.

Table IV. *KRAS* and *BRAF* gene mutations in human endometrial cancer-derived cell lines.

	KRAS		*BRAF*
	Codon 12 GCT(G)	Codon 13 GGC(G)	Codon 600 GTG(V)
Cell lines			
Hec108	GGT	GGC	GTG
SNG-II	GGT	GGC	GTG
Ishikawa	GGT	GGC	GTG
Hec-1B	GAT(D)	GGC	GTG
HHUA	GTT(V)	GGC	GTG
SNG-M	GTT(V)	GGC	GTG
HOOUA	GGT	GGC	GTG
KLE	GGT	GGC	GTG

Table V. Correlation of *KRAS* gene mutations with clinicopathological factors in cases of endometrial cancer.

	Grade		Stage		Age
	G1, 2	G3	I, II	III, IV	(average)
KRAS codon 12					
Mut	6	0	3	3	57.7
Wt	25	13	26	12	57
% Mut	19.4	0	10.3	20	
	p=0.1		p=0.32		p=0.88

Statistical analysis was performed with the χ^2 test and Mann-Whitney test. Mut, mutation; Wt, wild-type.

Table VI. Correlation of abnormal *BRAF* V600E genes with abnormal MMR and mutated *KRAS* genes.

	MSI		*hMLH1*		*KRAS* codon 12	
	Positive	Negative	M	U	Mut	Wt
BRAF						
Mut	0	0	0	0	0	0
Wt	17	27	17	27	6	38

MSI, microsatellite instability; M, methylated; U, unmethylated; Mut, mutation; Wt, wild-type.

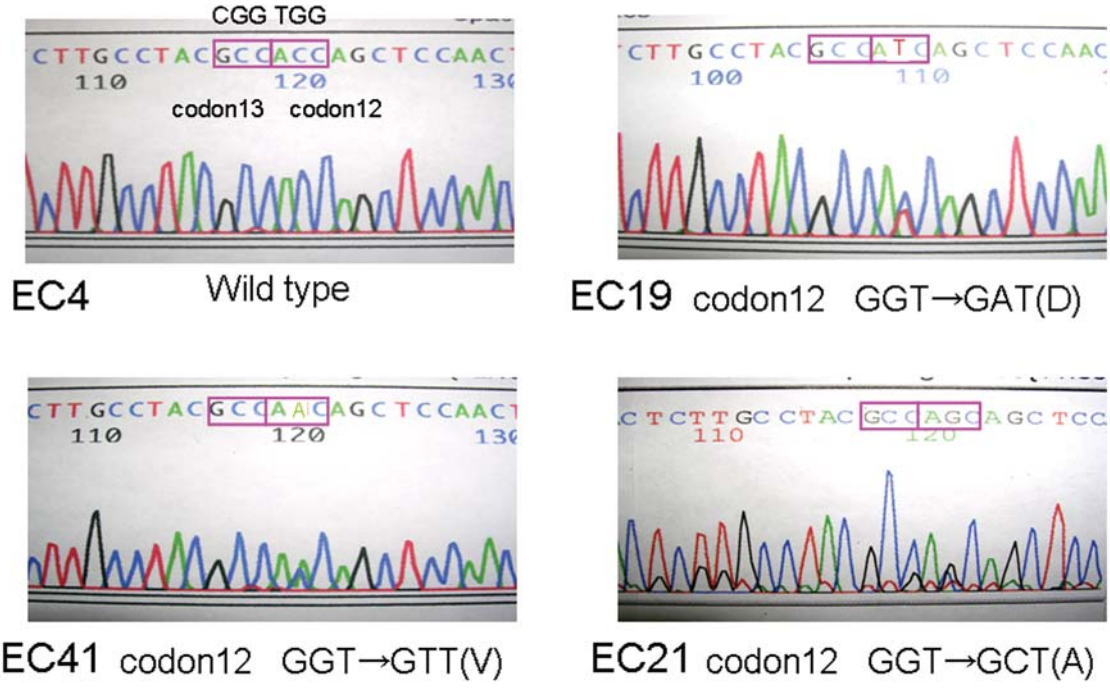

Figure 2. Analysis of point mutations of the *KRAS* gene in clinical samples of endometrial cancer. Three types of *KRAS* point mutation were detected at codon 12. No point mutation was observed at codon 13.

positive colon cancer have aberrant *hMLH1* hypermethylation (17). In our analysis, MSI-positive cases accounted for 38.6% of all cases of sporadic endometrial cancer. Mutch *et al* reported an incidence of MSI-positive cancer of 29% (18), with MSI occurring at higher rates in endometrial cancer than in colon cancer, suggesting that gene instability caused by an abnormal MMR gene is important in carcinogenesis of endometrial cancer. Our analysis showed aberrant *hMLH1* hypermethylation in 58.8% (10/17) of MSI-positive cases, with a significant positive correlation between aberrant *hMLH1* hypermethylation and MSI-positive cases of sporadic endometrial cancer (p=0.02). Based on this, we suggest that aberrant *hMLH1* hypermethylation causes MSI in endometrial cancer, as also seen in colon cancer.

Point mutations of the *KRAS* gene at codons 12 have been reported to occur in 0-46% of endometrial cancers and the most frequent codon 12 *KRAS* mutations are transitions from G to D, to V (19). Point mutations of the *KRAS* gene at codons 12 and 13 have been reported in 5.9% and 2.9% of patients with endometrial cancer, respectively, and the mutation showed a positive correlation with age upon development (20). Mutch *et al* found point mutations at codons 12, 13, and 61 of *KRAS* in 19.9%, 3.4% and 0.7% of cases of endometrial cancer, respectively, with a correlation with age upon development and a high rate of mutation in MSI-positive cases (18). In our analysis, point mutation at codon 12 was confirmed in 14% of cases, but none were observed at codon 13 and *KRAS* mutation showed no correlation with age. The incidence of well-differentiated adenocarcinoma tended to be high among cases with a mutation of *KRAS*, but the relationship was not significant, and there was no tendency for a higher rate of mutation of *KRAS* in MSI-positive cases. Point mutation of *KRAS* has been found in 51% of cases with colon cancer, and the rate in endometrial cancer is much lower (1). Mutation of *KRAS* may have some correlation with carcinogenesis in patients who develop sporadic endometrial cancer at an old age, but the current and previous results suggest that this mutation is not important for carcinogenesis in other cases of sporadic endometrial cancer.

Feng *et al* found mutation of the *BRAF* gene in 21% of cases of endometrial cancer, and proposed a correlation with decreased expression of the MMR gene (12). In contrast, Salvesen *et al* found the activating *BRAF* V600E mutation in only 2% of cases of endometrial cancer, and a consensus has not been obtained regarding the correlation between carcinogenesis of endometrial cancer and *BRAF* mutation (13). In our analysis, no *BRAF* V600E mutation was observed in cases of sporadic endometrial cancer. Collectively, these data suggested that the *BRAF* V600E mutation occurs at an extremely low rate in endometrial cancer, and thus may not be important for carcinogenesis of sporadic endometrial cancer. In contrast, the *BRAF* V600E mutation occurs at a high rate in sporadic colon cancer, and may be useful diagnostically to rule out the possibility of a hereditary tumor. However, this mutation is not useful in diagnosis of sporadic endometrial cancer.

Since we did not find a *BRAF* V600E mutation in our analysis, there was clearly no correlation between the *BRAF* V600E mutation and aberrant hypermethylation of *hMLH1* or MSI. Decreased expression of *hMLH1* due to aberrant hypermethylation could cause gene instability, with a high rate of mutation of a target gene such as *BRAF*. However, our results suggest that *BRAF* is not the target of abnormal MMR in sporadic endometrial cancer. On the other hand, since aberrant hypermethylation of *hMLH1* and MSI were detected at high rates in sporadic endometrial cancer patients, an abnormal MMR system is clearly associated with the mechanism of carcinogenesis in endometrial cancer. Identification of the new target gene for abnormal MMR will be extremely important for clarification of this mechanism.

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