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Overexpression of *p53*, Mutation of *hMLH1* and Microsatellite Instability in Gastric Carcinomas: Clinicopathologic Implications and Prognosis

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Purpose: Mutated *p53* is a tumor suppressor gene, *hMLH1* is a mismatch repair gene, and hypermethylation of *hMLH1* follows microsatellite instability (MSI). This research's aim is to investigate mutated *p53*, inactivated *hMLH1* and MSI in gastric cancer and their clinicopathologic implications.

Methods: Between 2003 and 2007, 618 patients underwent curative radical gastrectomy for gastric cancer at Seoul National University Bundang Hospital in Korea. We reviewed their medical charts and the pathologic reports with immunohistochemistry for *p53*, *hMLH1* and polymerase chain reaction for MSI in 509, 499, and 561 cases, respectively. These genetic markers were statistically compared with clinicopathologic features and postoperative survival.

Results: The expression ratios of mutated *p53*, inactivated *hMLH1*, and MSI were 32.8%, 8.4%, and 8.7%, respectively. Mutation of *p53* occurred more frequently in aged group (over 40), differentiated group (against the non-differentiated group), intestinal type, infiltrative type and positive lymph node metastasis group. Inactivated *hMLH1* occurred more frequently in aged group, differentiated group, intestinal type and expanding growth type group. MSI was found more frequently in aged group, intestinal type and expanding growth type group. All three genetic markers had no significant associations with the 5-year survival.

Conclusion: We identified significant relationships between mutated *p53*, inactivated *hMLH1*, and MSI with some clinicopathologic features of gastric cancer. However, there were no apparent relationships between *p53*, *hMLH1*, and MSI and prognosis. **(J Korean Surg Soc 2010;79:94-102)**

Key Words: Gastric cancer, *p53*, *hMLH1*, Microsatellite instability, Carcinogenesis

INTRODUCTION

Based on remarkable advances in recent molecular biologic analysis, it is known that gastric cancer is caused by a multistep accumulation of genetic alterations,(1) and various gene alterations exist in each step of carcinogenesis.(2) It is known that oncogenes, tumor suppressor genes, and replication error repair genes participate in that process. The most representative tumor suppressor gene is *p53*.(3) Mutations of *p53* are found most often in colorectal cancer; however, *p53* is found in several other kinds of cancers and also in stomach cancer with a high frequency.(4) *p53* repairs damaged DNA when abnormal cells enter into the S phase from the G1 phase, stops cell mitosis, and leads to apoptosis of cells when repair does not occur.(5) Many researchers have reported a role for mutated *p53* in stomach cancer; however, considerable differences have been shown regarding content, and strong evidence about the role as a prognostic factor has not been reported. Microsatellite refers to single sequence repeats of 1 or 6 units existing extensively in genes, and this site is

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where mismatch of bases occur during DNA replication. If there are abnormal mismatch repair genes, the length of the microsatellite will be lengthened or shortened. This phenomenon is referred to as microsatellite instability (MSI), and is also referred to as the mutator phenotype of an abnormal mismatch repair gene.(6) Mismatch repair genes are associated with *hMSH2*, *hMSH3*, and *hMLH1*.(7) Indeed, there is research that MSI is caused by inactivation of the *hMLH1* gene induced by hypermethylation.(8,9) In the current study, we investigated the mutated *p53*, inactivated *hMLH1* and MSI in gastric cancer and their clinicopathologic implications. And then we studied, whether these tree genetic markers affect the 5-year survival of gastric cancer patients.

METHODS

1) Patients and tissue samples

618 patients who underwent radical gastrectomy for gastric cancer at the Seoul National University Bundang Hospital between May 2003 and December 2005 were included in this study. We reviewed all the patients charts for their characteristics, clinicopathologic items and follow-up data. The median follow-up period was 36 months (1～60). Before surgery, informed consent was obtained from all participating patients. Mutated *p53* protein and inactivated *hMLH1* were evaluated by immunochemistry in 509 and 499 specimens, respectively (Fig. 1). And polymerase chain reaction (PCR) was done for MSI in 561 specimens. The pathologic analysis and reports after surgery was followed according to the UICC 6th.

2) Tissue array methods

Core tissue biopsies (2 mm in diameter) were obtained from individual paraffin-embedded gastric tumors (donor blocks) and arranged in a new recipient paraffin block (tissue array block) using a trephine apparatus (Super-biochips Laboratories, Seoul, Korea). An adequate case was defined as a tumor occupying ＞10% of the core area. As an internal control, each block contained normal gastric mucosa. Four-㎛-thick sections were cut from each tissue array block, deparaffinized, and dehydrated.

3) Immunohistochemistry

Immunohistochemical staining against mutated *p53* protein (1 : 100, mouse monoclonal antibody DO7; DAKO, Carpinteria, CA, USA) and *hMLH1* (dilution 1 : 50, Clone G168-728, 1㎍/ml; Pharmingen, San Diego, CA, USA) was performed using a streptavidin-biotin-peroxidase complex method after an antigen retrieval process using microwaves (3 times for 5 min each) for

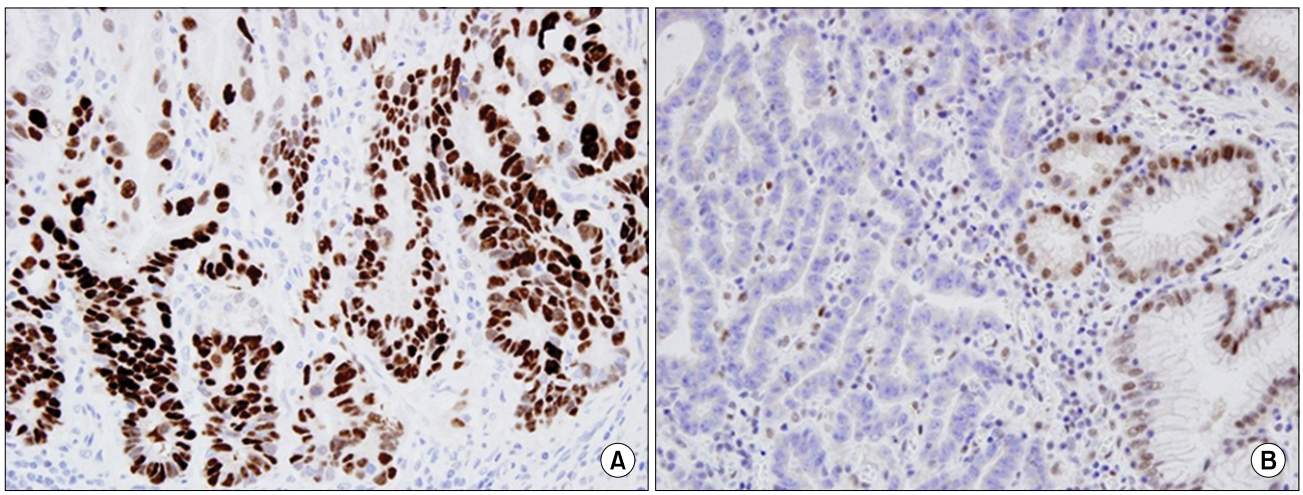

Fig. 1. Immunohistochemical detection of overexpression of *p53* (×400) (A). A gastric cancer case with complete loss of the protein in the invasive part (Left field) and preserved *hMLH1* expression in adjacent normal tissue (Right field) (×400) (B).

mutated *p53* protein, and using an autoclave for *hMLH1*. When >10% of cancer cells showed nuclear staining, we considered the case to be over-expression of the *p53* gene product or loss of *hMLH1* expression.

4) Microsatellite analysis

The DNA of cancerous tissue from 560 of 618 patients with consecutive gastric cancers was obtained from formalin-fixed, paraffin-embedded surgical blocks. The extracted DNA was amplified by PCR with fluorescent dye-labeled primers on two mononucleotide repeat microsatellite markers, BAT-26 and BAT-25 (located within intron 5 of the hMSH2 gene and introns of the c-kit oncogene, respectively). DNA was detected by a temperature-controlled DNA Sequencer (PRISM 377; Perkin-Elmer Corp., Foster City, CA, USA), and fragment analyses were carried out with Genscan software (Perkin-Elmer Corp.). MSI status was determined by size variation and the occurrence of additional bands in the PCR product from tumor DNA.

5) Statistical analysis

The χ^2-test was used to determine the statistical relationship between mutated *p53*, inactivation of *hMLH1*, MSI expression, and clinicopathologic characteristics. Survival curves were estimated using the Kaplan-Meier method, and the significance of differences between the survival curves was determined using the log-rank test. Multivariate survival analysis was performed using the Cox proportional hazards model. Statistical significance was defined as $P<0.05$. All statistical analyses were conducted using SPSS, version 15.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

1) Clinicopathologic features

The clinicopathologic features are summarized in Table 1. There were 411 male patients, and 207 female patients among the 618 cases. The majority of tumors were <5 cm in size (416 cases [67.2%]). The lower-third of the stomach was the most common location (326 cases [52.8%]).

Table 1. Clinicopathological features

	No. of patients (n=618) (%)
Sex	
Male	411 (66.5)
Female	207 (33.5)
Age	
Median (range)	58 (25~89)
Tumor size (cm)	
<5	416 (67.2)
5~10	168 (27.1)
≥10	34 (5.5)
Mean size (range)	4.69 (0.2~36)
Tumor location	
Lower	326 (52.8)
Middle	183 (29.6)
Upper	93 (15.0)
Entire	16 (2.6)
Histologic differentiation	
Well	98 (15.9)
Moderate	198 (32.0)
Poor	217 (35.1)
Signet ring cell	87 (14.1)
Mucinous	18 (2.9)
Lauren classification	
Intestinal	288 (46.4)
Diffuse	277 (44.8)
Mixed	53 (8.6)
Lymphatic invasion	
(−)	347 (56.1)
(+)	271 (43.8)
Perineual invasion	
(−)	432 (69.9)
(+)	186 (30.1)
Vascular invasion	
(−)	556 (90.0)
(+)	62 (10.0)
T stage	
T1	334 (54.0)
T2	168 (27.2)
T3	97 (15.7)
T4	19 (3.1)
N stage	
N0	356 (57.5)
N1	151 (24.4)
N2	55 (8.9)
N3	56 (9.0)
Stage	
Ia	288 (46.6)
Ib	98 (15.9)
II	87 (14.1)
IIIa	59 (9.5)
IIIb	8 (2.9)
IV	68 (11.0)

According to the WHO classification, the following histologic types were represented: well differentiated, 98 (15.9%); moderate differentiated, 198 (32.0%); poorly differentiated, 217 (35.1%); signet ring cell type, 87 (14.1%); and mucinous differentiated type, 18 (2.9%). Based on the Lauren classification, the intestinal type was found in 288 cases (46.4%), which was similar to 277 cases (44.8%) of the diffuse type. The distribution of tumor stages according to the UICC 6th was as follows: Ia, 288 (46.6%); Ib, 98 (15.9%); II, 87 (14.1%); IIIa, 59 (9.5%); IIIb, 18 (2.9%); and IV, 68 (11.0%). When classified by depth of invasion, 334 cases were early gastric cancer and 168 cases were advanced gastric cancer. 486 patients underwent a distal gastrectomy and 114 patients underwent a total gastrectomy.

2) Correlation between mutated *p53*, inactivated *hMLH1* and MSI

The association between the expression of mutated *p53* protein, inactivated *hMLH1*, and MSI is shown in Table 2. As mutated *p53* is more highly expressed, the probability of inactivation of *hMLH1* was decreased significantly (P=0.002), and the probability of detecting MSI was very small. When *hMLH1* gene is inactivated, there is a significant high probability (P=0.011) that MSI (instable) is found.

3) Clinicopathologic correlations with mutated *p53*, inactivated *hMLH1*, and MSI

The correlation between the expression of mutated *p53*, inactivated *hMLH1*, and MSI, and gender and age is shown in Table 3. The incidence of mutated *p53* was slightly higher in men, but there was no statistical significance. The incidence increased significantly as age increased. The group with increased mutated *p53* was older than the group without increased mutated *p53* by an average of 2.84 years. The group which inactivated *hMLH1* was also an average of 8.08 years older than the group without inactivated *hMLH1*. Comparing to the other group, the MSI-defined group was older by an average of 7.78 years.

The relationship between each genetic marker and tumor location is compared. Tumor location was based on the center of lesion, and the group of which cancer infiltrates entire stomach so cannot find that where the cancer was originated was excluded in order to compare the tendency of location effectively. The group of inactivated *hMLH1*, it occurred significantly more frequently in the lower-third (P=0.016). The group in which there was MSI occurred more frequently in the lower-third (P=0.001) too.

Regarding the relationship of each genetic marker with stage, lymph node (LN) metastasis, vascular invasion, neural invasion, lymphatic invasion, and overall stage is also described in Table 3. In the case of mutated *p53* over-expression, the probability of LN metastasis was significantly higher than that of wild type *p53* (P=0.025). However, no significant relationship was found between the other genetic markers and LN metastasis.

There were some clinical differences according to each genetic marker with respect to the WHO classification, Lauren's classification, and Ming' s criteria, as shown in Table 3. In the case of the WHO classification, for

Table 2. Correlation between mutated p53, inactivated hMLH1 and MSI

		p53 (n=509)		P-value*	*hMLH1* (n=499)		P-value*	MSI† (n=561)		P-value*
		(−) (%)	(+) (%)		Inactivated (%)	Activated (%)		Stable (%)	Instable (%)	
p53	(−)				37 (88.1)	298 (65.5)	0.002	293 (64.7)	37 (84.1)	0.002
	(+)				5 (11.9)	157 (34.5)		160 (35.3)	7 (15.9)	
hMLH1	Inactivated	37 (11.0)	5 (3.1)	0.002				7 (1.6)	35 (81.4)	0.011
	Activated	298 (89.0)	157 (96.9)					439 (98.4)	8 (18.6)	
MSI	Stable	293 (88.8)	160 (95.8)	0.002	7 (16.7)	439 (98.2)	0.011			
	Instable	37 (11.2)	7 (4.2)		35 (83.3)	8 (1.8)				

*The chi-square test was used to compare all variables; †MSI = microsatellite instability.

Table 3. Correlation between the expression of mutated *p53*, inactivated *hMLH1*, MSI and clinicopathologic features

	Mutated *p53* (n=509)		P-value*	*hMLH1* (n=499)		P-value*	MSI† (n=561)		P-value*
	Negative	Positive		Inactivated	Activated		Stable	Instable	
Sex			0.278			0.465			0.538
Male	225 (65.8)	115 (68.7)		29 (69.0)	306 (66.9)		342 (66.7)	33 (67.3)	
Female	117 (34.2)	52 (31.3)		13 (31.0)	151 (33.1)		170 (33.3)	16 (32.6)	
Age			**0.022**			**0.001**			**<0.001**
≤60	160 (46.8)	60 (35.9)		8 (19.0)	207 (45.3)		231 (45.1)	8 (16.3)	
>60 (n=351)	182 (53.2)	107 (64.1)		34 (81.0)	250 (54.7)		281 (54.9)	41 (83.7)	
Tumor location‡			0.960			**0.016**			**0.001**
Upper	55 (16.5)	25 (15.5)		2 (4.8)	73 (16.5)		83 (16.7)	2 (4.1)	
Middle	103 (30.9)	50 (31.1)		9 (21.4)	141 (31.8)		162 (32.6)	9 (18.4)	
Lower	175 (52.6)	86 (53.4)		31 (73.8)	229 (51.7)		252 (50.7)	38 (77.6)	
T stage			0.203			0.672			0.547
T1	187 (54.7)	81 (48.5)		19 (45.2)	249 (54.5)		284 (55.5)	26 (53.1)	
T2	90 (26.3)	57 (34.1)		15 (35.7)	126 (27.6)		133 (26.0)	16 (32.7)	
T3	55 (16.1)	27 (16.2)		7 (16.7)	71 (15.5)		82 (16.0)	7 (14.3)	
T4	10 (2.9)	2 (1.2)		1 (2.4)	11 (2.4)		13 (2.5)	0 (0.0)	
LN metastasis			**0.025**			0.404			0.237
(−)	201 (58.8)	82 (49.1)		25 (59.5)	257 (56.2)		291 (56.8)	31 (63.3)	
(+)	141 (41.2)	85 (50.9)		17 (40.5)	200 (43.8)		221 (43.2)	18 (36.7)	
Lymphatic invasion			0.421			0.074			0.104
(−)	187 (54.7)	89 (53.3)		18 (42.9)	255 (55.8)		294 (57.4)	23 (46.9)	
(+)	155 (45.3)	78 (46.7)		24 (57.1)	202 (44.2)		218 (42.6)	26 (53.1)	
Vascular invasion			0.317			0.603			0.281
(−)	305 (89.2)	152 (91.0)		38 (90.5)	412 (90.2)		461 (90.0)	46 (93.9)	
(+)	37 (10.8)	15 (9.0)		4 (9.5)	45 (9.8)		51 (10.0)	3 (6.1)	
Perineural invasion			0.295			0.419			0.367
(−)	232 (67.8)	118 (70.7)		28 (66.7)	317 (69.4)		358 (69.9)	36 (73.5)	
(+)	110 (32.2)	49 (29.3)		14 (33.3)	140 (30.6)		154 (30.1)	13 (26.5)	
Overall stage			0.539			0.351			0.309
Ia	162 (47.4)	65 (38.9)		17 (40.5)	211 (46.2)		242 (47.3)	23 (46.9)	
Ib	52 (15.2)	32 (19.2)		7 (16.7)	74 (16.2)		80 (15.6)	7 (14.3)	
II	51 (14.9)	25 (15.0)		11 (26.2)	62 (13.6)		70 (13.7)	12 (24.5)	
IIIa	31 (9.1)	20 (12.0)		3 (7.1)	45 (9.8)		49 (9.6)	4 (8.2)	
IIIb	8 (2.3)	5 (3.0)		1 (2.4)	10 (2.2)		13 (2.5)	1 (2.0)	
IV	38 (11.1)	20 (12.0)		3 (7.1)	55 (12.0)		58 (11.3)	2 (4.1)	
WHO criteria			**<0.001**			**0.012**			**0.032**
Differentiated	131 (38.3)	103 (61.7)		27 (64.3)	205 (44.9)		237 (46.3)	30 (61.2)	
Non-differentiated	211 (61.7)	64 (38.3)		15 (35.7)	252 (55.1)		275 (53.7)	19 (38.8)	
Lauren's type			**<0.001**			**0.004**			**0.010**
Intestinal	131 (41.2)	104 (69.8)		27 (73.0)	206 (48.8)		238 (50.2)	30 (69.8)	
Diffuse	187 (58.8)	45 (30.2)		10 (27.0)	216 (51.2)		236 (49.8)	13 (30.2)	
Ming's criteria			**0.014**			**0.011**			**<0.001**
Expanding	108 (31.6)	70 (41.9)		22 (52.4)	152 (33.3)		183 (35.7)	31 (63.3)	
Infiltrated	234 (68.4)	97 (58.1)		20 (47.6)	305 (66.7)		329 (64.3)	18 (36.7)	

*The chi-square test was used to compare all variables except mean age, which were compared using the unpaired t test; †MSI = microsatellite instability; ‡'Entire' was excluded in order to compare the tendency of location effectively. Bold = statistically significant P-values are indicated.

convenient comparison, well differentiation and moderate differentiation were combined in the differentiated group, and signet ring cell and poorly differentiated type were combined in the undifferentiated group. We then examined whether there was a difference in the extent of differentiation according to each genetic marker. In the

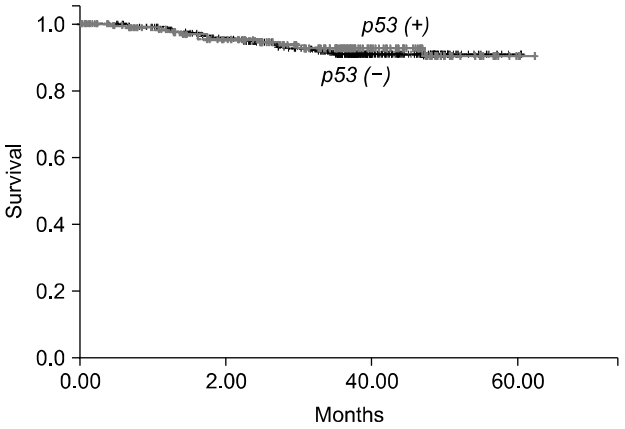

Fig. 2. Cumulative 5-year survival rates in 509 patients with gastric cancer according to the *p53* expression. There is no statistically significant difference of overall cumulative 5-year survival rates between *p53*(+) group and *p53*(−) group (P=0.807).

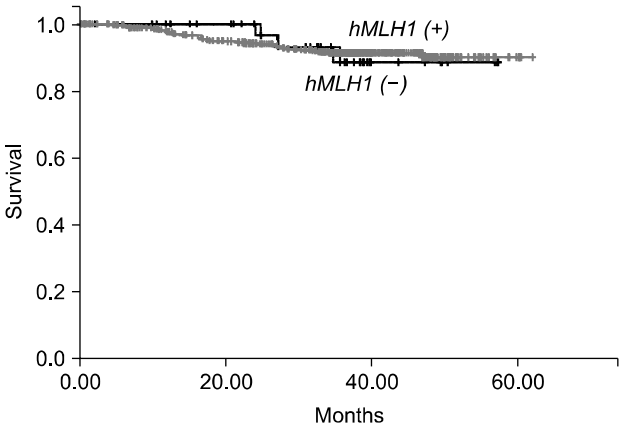

Fig. 3. Cumulative 5-year survival rates in 499 patients with gastric cancer according to the *hMLH1* activation. There is no statistically significant difference of overall cumulative 5-year survival rates between inactivated *hMLH1* group and activated *hMLH1* group (P=0.988).

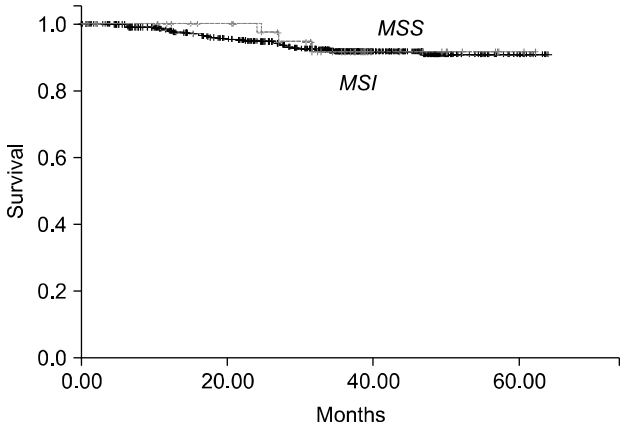

Fig. 4. Cumulative 5-year survival rates in 561 patients with gastric cancer according to the MSI expression. There is no statistically significant difference of overall cumulative 5-year survival rates between microsatellite instable group and microsatellite stable group (P=0.833). MSS = microsatellite stable, MSI = microsatellite instable.

Table 4. The univariate and multivariate analysis

Variables	Univariate (P-value)	Multivariate (P-value)
Tumor location	0.681	—
Tumor size	0.110	—
Tumor depth	0.004	0.490
Lymph node metastasis	0.048	0.979
TNM stage	0.000	0.000
WHO criteria	0.895	—
Lauren's classification	0.422	—
Ming's criteria	0.073	—
Lymphatic invasion	0.000	0.007
Vascular invasion	0.087	
Perineural invasion	0.001	0.004
Mutated *p53*	0.299	—
Inactivated *hMLH1*	0.436	—
MSI*	0.378	—

*MSI = microsatellite instability.

case of mutated *p53*, it was more significantly expressed in the group in which differentiation was better (P<0.001).

The mutated *p53* was more expressed in the group of intestinal type in according to Lauren's classification (P=0.001). According to Ming's criteria, there was slightly more expression of mutated *p53* in the group of expanding growth type (P=0.014). Differentiation was better in the group of inactivated *hMLH1* (P=0.012) than the group without inactivated *hMLH1*. The inactivation of *hMLH1* and MSI were more commonly detected in the intestinal type (P=0.004, P=0.010), and expanding growth pattern (P=0.011, P<0.001).

4) Survival analysis

Statistically significant variables were depth of tumor, LN metastasis, TNM stage, lymphatic invasion, and perineural invasion variables examined by univariate survival analysis. The over-expressed mutated *p53*, inactivation of *hMLH1*, and MSI was not related with survival significantly (Fig. 2～4). On multivariate analysis, TNM stage, lymphatic invasion and perineural invasion were remained

significantly associated with survival (Table 4).

DISCUSSION

With the recent advanced in molecular biology, many efforts to investigate the etiology of gastric cancer at the DNA level has been accomplished so that various oncogenes, inactivation of tumor suppressor genes, and growth factor deformation have been reported.(*1*) The unstable oncogenes are considered that playing a important role in malignant transformation of normal cells.(*10*) It is believed that they compose a signaling cascade required for cell mitosis and differentiation and protein engaging in gene regulator, and cell mitosis and differentiation of normal cell is inhibited by activation, so cancer occurs.(*11*) Expression of over-expressed mutated *p53* by using immunohistochemistry was observed in 167 patients among 509 patients who were analyzed, showing about 32.66% of expression rate. In gastric cancer patients, the frequency of the mutated *p53* protein by using immunohistochemistry has been reported in 30～60% from variable reports.(*12-14*) Regarding mutation of the *p53* with respect to age and tumor location, some studies suggested that there was the tendency that tumor occurred more in the upper-third, while mutation was less expressed in the age group <60 years of age, but there was no difference between the intestinal and diffuse types in histologic type.(*15*) The results of this study were the same with above research in that expression was increased. However, there was no statistical correlation with location, and a statistically significant expression was more in the intestinal type and expanding type. There is a report that expression of mutated *p53* protein was observed as 22% of the positive rate in early gastric cancer and 34% in advanced gastric cancer, especially high in well differentiated, but there isn't any linkage with tumor depth, LN metastasis, and vascular invasion.(*16*) In our study, most of results were the same as in the pre-mentioned study, but LN metastasis was detected more frequently in the group of mutated *p53* than group of wild type *p53*. Some studies wrote that the survival rate of gastric cancer patients having over-expression of mutated *p53* protein was very low since the danger of distant metastasis and LN metastasis was high.(*17,18*) Provided that *p53* is determined collectively with the other tumor markers, on the basis of that it was seemed to have a relation with differentiation and LN metastasis, it will be able to have clinical implication. There are a few researchers, who insist that mutated *p53* is related to a poor prognosis.(*13,17*) However, in our study, the mutated *p53* showed no significant influence to the 5-year survival. So we cannot conclude the meaning of mutated *p53* as an independent prognostic factor. Expression of MSI in gastric cancer tissue is variously reported (9.5～58.3%), and difference between each result is high.(*18-20*) These differences of expression rates according to researchers are considered that each researcher had used the different kind of markers or different number of markers. The frequency of MSI in this study was somewhat low (8.75%), which is attributed to using only BAT-26 and BAT-25 as a marker. It was also shown that the extent of the inactivated *hMLH1* gene was a similar value (8.43%); MSI was significantly related to *hMLH1* (P=0.011) This corresponds with the result of a prior study that the MSI phenotype of sporadic gastric cancers is mainly due to the inactivation of *hMLH1* by hypermethylation.(*21*) However mutated *p53* showed inverse relationship with MSI. As our results, there is a report that showed the significant inverse relationship between MSI and *p53* gene alterations in colon cancer.(*22*) They explained that there are two different molecular pathways to sporadic cancer; the microsatellite stable (but chromosomally unstable) pathway, probably initiated by APC mutations, and the MSI pathway. With respect to gastric cancer, clear agreement about the clinical meaning of MSI has not yet been compromised. Regarding the rate of expression of MSI according to age and tumor location, it was reported to be higher in the lower-third of gastric cancer in some existing studies,(*20,23*) and higher in the old age group in other studies.(*20,24*) However, Tamura et al.(*25*) insisted that MSI expression was frequently observed at tumors located in the cardia, and it was not related to age, gender, and histologic differentiation. In our research, the rate of

expression was high in older age and gastric cancer occurred in the lower-third of the stomach. There are some suppositions that an increase of an imbalance between DNA methyltransferase and demethylase activities with age may be responsible for *hMLH1* hypermethylation.(*26*) But the mechanism has not been explained, clearly. The rate of expression was high in intestinal type and expanding growth type of gastric cancer so that our research's results were different from that of Tamura et al. Generally, the prognosis of tumor having MSI is good,(*27,28*) and the reason for a good prognosis is that T-cell immune reaction against mutated protein is increased in tumor having mutator phenotype.(*28*) As to gastric cancer, there are some other studies that have also reported a good prognosis of gastric cancer having expression of MSI,(*23,24*) however we could not find a statistically significant relationship between MSI expression in gastric cancer and prognosis.

In conclusion, in this research, we couldn't find any possibility for the independent prognostic factors about *p53*, *hMLH1* and MSI. But three genetic markers are correlated significantly with some clinicopathologic factors that can affect prognosis, like tumor differentiation, type and especially LN metastasis. This means that these genetic markers can affect the tumor aggressiveness. So if further studies are followed, detection of these genetic markers can be helpful for tailored treatment plan for each patient.

REFERENCES

1) Correa P. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. Cancer Res 1992;52:6735-40.
2) Tahara E. Molecular mechanism of stomach carcinogenesis. J Cancer Res Clin Oncol 1993;119:265-72.
3) Harris AL. Mutant p53--the commonest genetic abnormality in human cancer? J Pathol 1990;162:5-6.
4) Hollstein MC, Peri L, Mandard AM, Welsh JA, Montesano R, Metcalf RA, et al. Genetic analysis of human esophageal tumors from two high incidence geographic areas: frequent p53 base substitutions and absence of ras mutations. Cancer Res 1991; 51:4102-6.
5) Yonish-Rouach E, Resnitzky D, Lotem J, Sachs L, Kimchi A, Oren M. Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. Nature 1991;352:345-7.
6) Woerner SM, Kloor M, von Knebel Doeberitz M, Gebert JF. Microsatellite instability in the development of DNA mismatch repair deficient tumors. Cancer Biomark 2006;2:69-86.
7) Thibodeau SN, French AJ, Roche PC, Cunningham JM, Tester DJ, Lindor NM, et al. Altered expression of hMSH2 and hMLH1 in tumors with microsatellite instability and genetic alterations in mismatch repair genes. Cancer Res 1996;56:4836-40.
8) Fleisher AS, Esteller M, Wang S, Tamura G, Suzuki H, Yin J, et al. Hypermethylation of the hMLH1 gene promoter in human gastric cancers with microsatellite instability. Cancer Res 1999;59:1090-5.
9) Rhyu MG, Park WS, Meltzer SJ. Microsatellite instability occurs frequently in human gastric carcinoma. Oncogene 1994;9:29-32.
10) Peddanna N, Holt S, Verma RS. Genetics of gastric cancer. Anticancer Res 1995;15:2055-64.
11) Correa P, Shiao YH. Phenotypic and genotypic events in gastric carcinogenesis. Cancer Res 1994;54(7 Suppl):1941s-3s.
12) Starzynska T, Markiewski M, Domagala W, Marlicz K, Mietkiewski J, Roberts SA, et al. The clinical significance of p53 accumulation in gastric carcinoma. Cancer 1996;77:2005-12.
13) Martin HM, Filipe MI, Morris RW, Lane DP, Silvestre F. p53 expression and prognosis in gastric carcinoma. Int J Cancer 1992;50:859-62.
14) Ku KB, Park SH, Chung HY, Yu W, Lee MH. p53 Mutation and p53 protein expression in gastric cancer tissue. J Korean Surg Soc 2007;72:283-9.
15) Rugge M, Shiao YH, Busatto G, Cassaro M, Strobbe C, Russo VM, et al. The p53 gene in patients under the age of 40 with gastric cancer: mutation rates are low but are associated with a cardiac location. Mol Pathol 2000;53:207-10.
16) Uchino S, Noguchi M, Ochiai A, Saito T, Kobayashi M, Hirohashi S. p53 mutation in gastric cancer: a genetic model for carcinogenesis is common to gastric and colorectal cancer. Int J Cancer 1993;54:759-64.
17) Monig SP, Eidt S, Zirbes TK, Stippel D, Baldus SE, Pichlmaier H. p53 expression in gastric cancer: clinicopathological correlation and prognostic significance. Dig Dis Sci 1997;42: 2463-7.
18) Lee HS, Choi SI, Lee HK, Kim HS, Yang HK, Kang GH, et al. Distinct clinical features and outcomes of gastric cancers with microsatellite instability. Mod Pathol 2002;15:632-40.
19) Liu P, Zhang XY, Shao Y, Zhang DF. Microsatellite instability in gastric cancer and pre-cancerous lesions. World J Gastroenterol 2005;11:4904-7.
20) Oh SH, Choi YK, Hong KH, Kim SH, Paik NH, Yang YI, et al. Microsatellite instability and overexpression of p53 protein in human gastric carcinomas: clinicopathologic implications and prognosis. J Korean Surg Soc 2000;59:206-22.
21) Leung SY, Yuen ST, Chung LP, Chu KM, Chan AS, Ho JC.

hMLH1 promoter methylation and lack of hMLH1 expression in sporadic gastric carcinomas with high-frequency microsatellite instability. Cancer Res 1999;59:159-64.
22) Samowitz WS, Holden JA, Curtin K, Edwards SL, Walker AR, Lin HA, et al. Inverse relationship between microsatellite instability and K-ras and p53 gene alterations in colon cancer. Am J Pathol 2001;158:1517-24.
23) Wu MS, Lee CW, Shun CT, Wang HP, Lee WJ, Chang MC, et al. Distinct clinicopathologic and genetic profiles in sporadic gastric cancer with different mutator phenotypes. Genes Chromosomes Cancer 2000;27:403-11.
24) Seruca R, Santos NR, David L, Constancia M, Barroca H, Carneiro F, et al. Sporadic gastric carcinomas with microsatellite instability display a particular clinicopathologic profile. Int J Cancer 1995;64:32-6.
25) Tamura G, Sakata K, Maesawa C, Suzuki Y, Terashima M, Satoh K, et al. Microsatellite alterations in adenoma and differentiated adenocarcinoma of the stomach. Cancer Res 1995;55:1933-6.
26) Nakajima T, Akiyama Y, Shiraishi J, Arai T, Yanagisawa Y, Ara M, et al. Age-related hypermethylation of the hMLH1 promoter in gastric cancers. Int J Cancer 2001;94:208-11.
27) dos Santos NR, Seruca R, Constancia M, Seixas M, Sobrinho-Simoes M. Microsatellite instability at multiple loci in gastric carcinoma: clinicopathologic implications and prognosis. Gastroenterology 1996;110:38-44.
28) Bodmer W, Bishop T, Karran P. Genetic steps in colorectal cancer. Nat Genet 1994;6:217-9.