High Frequency of Microsatellite Instability in Intestinal-type Gastric Cancer in Korean Patients

Won Hyuk Choe, M.D., Sun-Young Lee, M.D., Jun Haeng Lee, M.D., Sang Goon Shim, M.D., Young-Ho Kim, M.D., Poong-Lyul Rhee, M.D., Jong Chul Rhee, M.D., Chang-Seok Ki, M.D., Jong-Won Kim M.D., Sang Yong Song M.D. and Jae J. Kim M.D.

Departments of Internal Medicine, Laboratory Medicine and Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Background: Although there have been some reports on microsatellite alterations in gastric cancer, findings are inconsistent regarding the associations between histological classification and microsatellite instability (MSI). In the present study, we attempted to determine whether Lauren's histological subtypes are related with MSI status.

Methods: Paraffin-embedded tissue samples from 14 diffuse-type and 14 intestinal-type gastric adenocarcinomas were matched up according to patient gender and age. Mononucleotide markers (BAT25 and BAT26) and dinucleotide markers (D2S123, D5S346, and D17S250) were used for MSI analyses. Microsatellite genotypes were categorized in terms of high MSI incidence (MSI-H, >30% positive marker) or low MSI incidence (MSI-L, <30% positive marker). Losses of hMLH1 and hMSH2 protein expression were immunohistochemically studied.

Results: MSI-H was observed in 11 cases (78%) of the 14 intestinal-type cases as compared to 3 (21%) of the 14 diffuse-type cases (p=0.007). In MSI-H tumors, 10 cases (71%) showed losses of hMLH1 protein expression, while 2 cases (14%) in MSI-L tumors showed losses of hMLH1 protein expression (p=0.006).

Conclusion: MSI-H tumors are more frequently found in intestinal-type gastric cancer, which suggests the possibility that there are different pathogenic pathways in gastric carcinogenesis according to histologic type.

Key Words: Gastric cancer, Histology, Microsatellite instability

INTRODUCTION

Gastric adenocarcinoma is one of the most frequently observed malignant tumors in the world, contributing significantly to cancer mortality, especially on the Asian continent. Recently, it became clear that gastric carcinogenesis is a multifactorial, multi-step process requiring sequential alterations in genes which codify for tumor suppressors, proto-oncogenes, gate-keeper genes, enzymes, growth factors, and membrane or nuclear receptors (the multi-hit hypothesis). Among these, mutation carriers of DNA mismatch repair genes exhibit a characteristic phenotype termed microsatellite instability (MSI), which is characterized by an accelerated accumulation of single nucleotide mutations and of alterations in the lengths of simple repetitive microsatellite sequences found throughout the genome. In the past, MSI was considered to be restricted to the field of colorectal cancer. However, it has recently been reported that sporadic cancers, including gastric cancer, are also related with MSI. In Korea, several studies have suggested that susceptibility to gastric cancer is caused by mutations in one of the genes in the DNA mismatch repair system.

Gastric adenocarcinoma is classified as intestinal- or diffuse-type according to histologic characteristics. Intestinal-type adenocarcinomas are usually located in the distal stomach and...
Table 1. Primer sequences of the microsatellite instability (MSI) markers

| Marker  | Size (bp) | Dye   | Primer Sequences                                      |
|---------|-----------|-------|-------------------------------------------------------|
| BAT25   | 90        | Dye A¹ | 5’-TCG CCT CCA AGA ATG TAA GT-3’                     |
|         |           |       | 5’-TCT GCA TTT TAA CTA TGG CTC-3’                     |
| BAT26   | 80-100    | Dye B² | 5’-TGA CTA CTT TTG ACT TCA GCC-3’                     |
|         |           |       | 5’-AAC CAT TCA ACA TTT TTA ACC-3’                     |
| D2S123  | 197-227   | Dye A¹ | 5’-AAA CAG GAT GCC TGC CTT TA-3’                      |
|         |           |       | 5’-GGA CTCT TCC ACC TAT GGG AC-3’                     |
| D5S346  | 96-122    | Dye C³ | 5’-ACT CAC TCT AGT GAT AAA TCG G-3’                    |
|         |           |       | 5’-AGC AGA TAA GAC AGT ATT ACT AGT T-3’                |
| D17S250 | 150-169   | Dye A¹ | 5’-GGA AGA ATC AAA TAG ACA AT-3’                       |
|         |           |       | 5’-GCT GGC CAT ATA TAT ATT TAA ACC-3’                  |

¹Dye A, 6-carboxyfluorescin; ²Dye B, 6-carboxy-4, 7, 2’, 4’, 5’, 7’-hexachlorofluorescein; ³Dye C, 2, 7’, 8’-benzo-5’-fluoro-2’, 4, 7-trichloro-5’-carboxyfluorescein

possess a characteristic glandular structure that is believed to arise from the intestinal metaplastic epithelium. In contrast, diffuse-type adenocarcinomas especially invade the cardia and have poor glandular formations that are believed to arise from gastric mucous cells. These two different types are considered to develop through different molecular pathways, raising the possibility that they may have different genetic background characteristics, such as MSI rates.

Although previous studies have reported variable MSI rates in the two major types of histological gastric cancer, findings have been inconsistent regarding the association of MSI with these two pathological features. Most reports have suggested that MSI is more frequently observed in the intestinal type, but, some have also reported the opposite finding. These different results regarding MSI and intestinal/diffuse histotype may reflect ethnic, racial, or geographic differences, in addition to discrepancies due to different definitions of MSI cancer. Moreover, due to the lack of well-defined criteria for microsatellite analysis, different numbers or types of markers were used in each study. Recently, it has been reported that MSI was more frequently seen in gastric cancer from Korea.

Therefore, we investigated MSI in gastric cancer in Korea according to Lauren’s classification.

MATERIALS AND METHODS

Patients

A total of 28 gastric adenocarcinoma patients were retrieved among the gastric cancer patients who underwent gastrectomy in 1996 at Samsung Medical Center, Seoul, Korea. Fourteen cases of cardiac adenocarcinoma were selected first, and another 14 cases of antral adenocarcinoma were selected, matching for age and gender. None of the patients had any previous history of malignancy. The postoperative stages varied from stage IB to stage IV.

DNA preparation

Serial gastric sections from cancer tissue and adjacent noncancerous tissue were obtained at 5 µm thickness and stained with hematoxylin and eosin. Only tissues containing more than 80% of cancer tissue were deemed acceptable for microsatellite analysis. Genomic DNA from tumors and from corresponding normal tissue were obtained from paraffin blocks by microdissection. Deparaffinization was done by xylene for 20 minutes and 40 minutes at room temperature. Rehydration was performed by washing in 100%, 95%, 80%, and 70% ethanol for 10 minutes separately, at room temperature. Tumor tissue was separated from normal tissue with a needle, and was then inserted into an Eppendorf tube. DNA was extracted from the microdissected tissues using 200 µL of proteinase K solution (190 µL of protein kinase digestion buffer with 10 µL of 10 mg/mL proteinase K) in lysis buffer containing 0.5% Tween-20, 1M Tris (pH 8.5), and 500 mM EDTA (pH 8.0). Tissues were incubated overnight in lysis buffer solution at 55°C. After the overnight incubation, centrifugation was performed at 14,000 rpm for 15 minutes at 4°C.

Analysis of MSI

MSI was analyzed by PCR amplification with fluorescent dye-labeled primers of mononucleotide markers (BAT25 and BAT26) and dinucleotide markers (D2S123, D5S346, and D17S250) specific for the microsatellite loci. Primer sequences of the MSI markers are shown in table 1. As previously described, PCR was performed over 35 cycles of: 1 minute at 94°C, 1 minute at 55°C, and 1 minute at 72°C for the BAT25 and BAT26 primers. For D2S123, PCR was performed over 35 cycles of: 30 seconds at 94°C, 1 minute at 54°C, and 1 minute at 72°C. For D5S346, PCR was performed over 35 cycles of: 30 seconds at 94°C, 30 seconds at 55°C, and 30
Table 2. Clinicopathological findings in high microsatellite instability (MSI-H) and low microsatellite instability (MSI-L) cases

|                  | MSI-H | MSI-L | p-value |
|------------------|-------|-------|---------|
| Male : Female    | 8 : 6 | 10 : 4| NS      |
| Age (mean ± SD)  | 66.7±13.0 | 67.9±12.5 | NS |
| Intestinal : Diffuse* | 11 : 3 | 3 : 11 | 0.007 |
| Antrum : Cardia  | 9 : 5 | 5 : 9 | NS |
| Stage            |       |       |         |
| IB               | 1     | 1     | NS      |
| II               | 7     | 2     |         |
| IIIA             | 2     | 2     |         |
| IIIB             | 4     | 6     |         |
| IV               | 0     | 3     |         |

*Lauren’s classification; † MSI-H, >30% positive microsatellite instability marker; ‡ MSI-L, <30% positive microsatellite instability marker

Figure 1. Detection of MSI by analysis of microsatellite markers in tumors and in corresponding normal tissue. Mutant alleles are indicated with arrows in each tumor trace: (A) BAT25, (B) BAT26, (C) D2S123, and (D) D17S250.

Electropherograms were analyzed independently by two different investigators. MSI was defined as a band shift in either of the two alleles or in the appearance of a differently-sized band in the tumor sample (Figure 1). Microsatellite genotypes were categorized as a high incidence of MSI (MSI-H) when instability was detected in more than 30% of markers and as a low incidence of MSI (MSI-L) when instability was detected in less than 30% of markers.

Immunohistochemical staining and analysis

Immunohistochemical staining for hMLH1 and hMSH2 protein was performed as previously described. Losses of hMLH1 and hMSH2 protein expressions were determined by immunohistochemical staining. Antibody to hMLH1 (SC-581; Santa Cruz...
Biotechnology INC, CA, USA), a rabbit polyclonal antibody, was prepared with a full-length *hMLH1* protein. Another rabbit polyclonal antibody, antibody to *hMSH2* (SC-494: Santa Cruz Biotechnology INC, CA, USA), was generated via a COOH terminal fragment of *hMSH2* protein. As previously described, LOVO cells, which express *hMLH1*, were stained simultaneously as a positive control.

Statistics

The possible association between MSI status and histology was assessed using Fisher's exact test. The chi-square test and Fisher's exact test were used for statistical assessment of the association between MSI status and clinicopathological profiles. The Kaplan-Meier method was used to estimate the survival probability as a function of time. The log-rank test (generalized Wilcoxon's test) was performed in order to analyze the differences in patient survival. A *p*-value less than 0.05 was accepted as statistically significant.

Results

Of the 28 cases, 14 cases (50%) manifested as MSI-H, while the other 14 cases were classified as MSI-L. Among the MSI-L cases, 8 showed no MSI (Table 2). MSI-H was more frequently observed in adenocarcinomas of the intestinal-type (11/14) than in those of the diffuse-type (3/14) (78% and 21%, respectively, *p*=0.007). There was no statistically significant correlation between MSI status and sex, age, location of the tumor, or tumor stage (Table 2). According to each of the markers, there was no significant difference between the intestinal-type and the diffuse-type in *BAT25* (*p*=0.42), *BAT26* (*p*=1.00), *D2S123* (*p*=0.26), *D5S346* (*p*=0.21), and *D17S250* (*p*=0.24).

Immunohistochemical staining results on two mismatch repair proteins revealed strong correlations with MSI status (Figure 2). In MSI-H tumors, 10 cases (71%) showed losses of *hMLH1*
protein expression and 3 cases (21%) showed losses of hMSH2 protein expression. In MSI-L tumors, 2 cases (14%) showed losses of hMLH1 protein expression and 1 case (7%) showed a loss of hMSH2 protein expression (Figure 3). A loss of hMLH1 protein expression was significantly correlated with MSI status (p=0.006).

After gastrectomy, 8 patients among 14 MSI-H cases and 7 patients among 14 MSI-L cases underwent adjunctive chemotherapy with or without radiotherapy. Follow-up intervals after surgical resection ranged from 73 to 102 months with a median of 87 months in 10 patients who were alive throughout follow-up. Furthermore, follow-up times ranged from 3 to 34 months with a median of 15 months in 18 patients who died of gastric cancer. Using the Kaplan-Meier method, the survival curves of patients at all stages were plotted using MSI status (Figure 4). The MSI-L group exhibited lower survival, whereas the MSI-H group revealed better survival. However, there was no statistically significant difference between the MSI-H group and the MSI-L group in cumulative survival (p=0.17).

**DISCUSSION**

In the present study, MSI was observed more frequently than in previous reports, and MSI-H was more frequently observed in the intestinal-type than in the diffuse-type adenocarcinomas. This discrepancy is probably related to the definition and the methods of describing MSI, the patient population that underwent evaluation, and our small sample size. In spite of this unexpected data, our report is supported by previous studies which reported that MSI is more frequently observed in the intestinal-type than in the diffuse-type gastric adenocarcinoma. Moreover, a previous study concluded that MSI is associated with the intestinal histological type and chromosomal deletion, which in turn lead to an increase in alterations of cancer-related genes. This close relationship between MSI and intestinal-type gastric cancer may also suggest a genetic model common to colon and gastric cancers. Intestinal metaplasia has been considered a precancerous lesion of intestinal-type gastric carcinoma, and MSI-associated mutations were detected exclusively in both intestinal-type gastric carcinomas and colon cancers. Thus, these two cancers appear to be closely related to each other histopathologically, as well as genetically.

However, these findings are inconsistent in regards to the association between MSI and these two pathological features. Replication error-positive phenotypes were more frequently observed in scirrhous-type gastric cancer (75%) than in other histologic types, which suggests that scirrhous-type gastric cancers may have germline gene mutations in their DNA mismatch repair systems, such as hMSH2 or hMLH1. Moreover, MSI was detected in 57% of the foveolar-type and 8% of intestinal-type. These findings suggest that foveolar-type tumors contain several histopathological problems and are prone to losing their glandular structure and progressing to undifferentiated-type tumors. Thus, they should be regarded as precursors of undifferentiated-type tumors. They concluded that the ‘mutator pathway’, characterized by MSI, plays an important role in the tumorigenesis of foveolar-type tumors, but not in the complete-type intestinal metastatic phenotype. In addition to discrepancies due to different definitions of MSI cancer, the different results in terms of the MSI and intestinal/diffuse histotype may reflect ethnic, racial, or geographic differences.

The aforementioned study demonstrated that there appear to be three different profiles of carcinogenesis: 1) p53 mutations which accompany the onset of dysplasia and intestinal-type carcinoma; 2) alterations of E-cadherin, both with regards to mutations and abnormal expression; and 3) DNA repair mechanism alterations which condition microsatellite instability seem mutually exclusive with regards to p53 mutations. These alterations are correlated with anturally located intestinal-type carcinoma, with little metastatic tendency and a better prognosis. Some studies have reported that RER-positive cases mostly consisted of intestinal tumors and have been shown to carry relatively good prognoses. In the present study, the MSI-H group tended to have better survival and a more favorable prognosis, but this finding was not statistically significant. Although there exists a report which suggests the irrelevance between survival and MSI status, our result, like that of previous studies, indicates that MSI seems to improve survival. Unfortunately, we were unable to statistically demonstrate it because the number of patients was too small.

Immunohistochemical staining is a consistent element in the study of MSI. A previous study demonstrated that immunohistochemistry could accurately discriminate between MSI-H and microsatellite stable tumors. Moreover, the majority of germline mutations have been found in key MMR proteins, i.e., hMLH1 and hMSH2 proteins. In the present study, immunostaining for the loss of hMLH1 protein expression revealed a significant correlation between its loss and MSI status (p=0.006), suggesting that promoter hypermethylation of hMLH1 might be correlated with a loss of hMLH1 protein expression, which results in MSI, especially in intestinal-type gastric adenocarcinoma.

In summation, MSI was more observed more frequently in adenocarcinomas of the intestinal-type. This suggests that the intestinal- and diffuse-types of gastric adenocarcinoma, by Lauren’s classification, are associated with different molecular carcinogenic pathways. Furthermore, our results suggest the
importance of the hMLH1 promoter in causing MSI-H gastric cancer, and imply that the loss of hMLH1 protein expression is related with intestinal-type gastric adenocarcinoma. Moreover, our results indicate that hMLH1 protein expression analysis should be considered for the assessment of MSI-H status.

REFERENCES

1) Correa P. Human gastric carcinogenesis: a multistep and multifactorial process. Cancer Res 52:6726-6740, 1992
2) Seregini E, Ferrari L, Martinterri A, Bombardieri E. Diagnostic and prognostic tumor markers in the gastrointestinal tract. Semin Surg Oncol 20:147-166, 2001
3) Aaltonen LA, Peltomaki P, Leach FS, Sistonen P, Pylkkanen L, Mecklin JP, Jarvinen H, Powell SM, Jen J, Hamilton SR, Petersen GM, Kinzler KW, Vogelstein B, Chapellle A. Clues to the pathogenesis of familial colorectal cancer. Science 260:812-816, 1993
4) Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Peruchio M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature 363:558-561, 1993
5) Strickler JG, Zheng J, Shu Q, Burgart LJ, Alberts SR, Shibata D. p53 mutations and microsatellite instability in sporadic gastric cancer: when guardians fail. Cancer Res 54:4750-4755, 1994
6) Halling KC, Harper J, Moskaluk CA, Thibodeau SN, Petroni GR, Yustein AS, Tosi P, Minacci C, Roviello F, Piva P, Hamilton SR, Jackson CE, Powell SM. Origin of microsatellite instability in gastric cancer. Am J Pathol 155:205-211, 1999
7) Hayden JD, Martin IG, Cawkwell L, Quirke P. The role of microsatellite instability in gastric carcinoma. Gut 42:300-303, 1998
8) Semb S, Yokozaki H, Yamamoto S, Yasui W, Tahara E. Microsatellite instability in precancerous lesions and adenocarcinomas of the stomach. Cancer 77:1620-1627, 1996
9) Kim HG, Yang JH, Choi BR, Lee CH, Bae JD, Cho CH, Chung WB, Hong SH, Kim JA, Kim JW, Sohn YK. Microsatellite instability in gastric adenocarcinoma tissue obtained by endoscopic biopsy. Korean J Gastroenterol 35:597-599, 2000
10) Choi SW, Choi JR, Chung YJ, Kim KM, Rhyu MG. Prognostic implications of microsatellite genotypes in gastric carcinoma. Int J Cancer 89:379-383, 2000
11) Kang YH, Bae SI, Kim WH. Comprehensive analysis of promoter methylation and altered expression of HMLH1 in gastric cancer cell lines with microsatellite instability. J Cancer Res Clin Oncol 128:119-124, 2002
12) Kim HS, Lee BL, Wook DK, Bae SI, Kim WH. Assessment of markers for the identification of microsatellite instability phenotype in gastric neoplasms. Cancer Lett 164:61-68, 2001
13) Kim KM, Kwon MS, Hong SJ, Min KO, Seo EJ, Lee KY, Choi SW, Rhyu MG. Genetic classification of intestinal-type and diffuse-type gastric cancers based on chromosomal loss and microsatellite instability. Virchows Arch 443:491-500, 2003
14) Kim JJ, Baek MJ, Kim L, Kim NG, Lee YC, Song SY, Noh SH, Kim H. Accumulated frameshift mutations at coding nucleotide repeats during the progression of gastric carcinoma with microsatellite instability. Lab Invest 79:1113-1120, 1999
15) Lim S, Lee HS, Kim HS, Kim YJ, Kim WH. Alteration of E-cadherin-mediated adhesion protein is common, but microsatellite instability is uncommon in young age gastric cancers. Histopathology 42:128-136, 2003
16) Sepulveda AR, Santos AC, Yamaoka Y, Wu L, Gutierrez O, Kim JG, Graham DY. Marked differences in the frequency of microsatellite instability in gastric cancer from different countries. Am J Gastroenterol 94:3334-3338, 1999
17) Chung YJ, Kim KM, Choi JR, Choi SW, Rhyu MG. Relationship between intratumor histological heterogeneity and genetic abnormalities in gastric carcinoma with microsatellite instability. Int J Cancer 82:782-788, 1999
18) Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma an attempt at a histo-clinical classification. Acta Pathol Microbiol Scand 64:31-49, 1965
19) Correa P, Shiao YH. Phenotypic and genotypic events in gastric carcinogenesis. Cancer Res 54:1941s-1945s, 1994
20) Solcia E, Fiocca R, Luzzetti O, Villani L, Padovan L, Calistri D, Ranzani GN, Chiariavalli A, Capella C. Intestinal and diffuse gastric cancers arise in a different background of Helicobacter pylori gastritis through different gene involvement. Am J Surg Pathol 20:58-62, 1996
21) 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 110:38-44, 1996
22) Chung YJ, Song JM, Lee JY, Jung YT, Soo EJ, Choi SW, Rhyu MG. Microsatellite instability associated mutations associate preferentially with the intestinal type of primary gastric carcinomas in a high risk population. Cancer Res 56:4662-4665, 1996
23) Buonsanti G, Calistri D, Padovan L, Luzzetti O, Fiocca R, Solcia E, Ranzani GN. Microsatellite instability in intestinal– diffuse-type gastric carcinoma. J Pathol 182:167-173, 1997
24) Fiocca R, Luzzetti O, Villani L, Mastracci L, Quillici P, Grillo F, Ranzani GN. Molecular mechanisms involved in the pathogenesis of gastric carcinoma: interactions between genetic alterations, cellular phenotype and cancer histotype. Hepatogastroenterology 48:1522-1530, 2001
25) Wu MS, Lee CW, Shun CT, Wang HP, Lee WJ, Sheu JC, Lin JT. Clinicopathological significance of altered loci of replication error and microsatellite instability–associated mutations in gastric cancer. Cancer Res 58:1494-1497, 1998
26) Endoh Y, Tamura G, Aijoka Y, Watanabe H, Motoyama T. Frequent hypemethylation of the MLH1 gene promoter in differentiated-type tumors of the stomach with the gastric foveolar phenotype. Am J Pathol 157:717-722, 2000
27) Endoh Y, Seka K, Tamura G, Ohmura K, Aijoka Y, Watanabe H, Motoyama T. Cellular phenotypes of differentiated-type adenocarcinomas and precancerous lesions of the stomach are dependent on the genetic pathways. J Pathol 191:257-262, 2000
28) Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt NW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, 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 58:5243-5247, 1998
29) Kang GH, Shin YH, Po JY. Correlation of methylation of the MLH1 promoter with lack of expression of hMLH1 in sporadic gastric carcinomas with replication error. Lab Invest 79:933-939, 1999
30) Rhyu MG. Genetic events underlying morphological complexity of...
gastric carcinoma. J Korean Med Sci 13:339-349, 1998
31) Luinetti O, Fiocca R, Villani L, Alberizzi P, Ranzani GN, Solcia E. Genetic pattern, histological structure and cellular phenotype in early and advanced gastric cancers: evidence for structure-related genetic subsets and for loss of glandular structure during progression of some tumors. Hum Pathol 29:702-709, 1998
32) Choi SW, Choi JR, Chung YJ, Kim KM, Rhyu MG. Prognostic implications of microsatellite genotypes in gastric carcinoma. Int J Cancer 89:379-383, 2000
33) Schneider BG, Bravo JC, Roa JC, Roa I, Kim MC, Lee KM, Plaisance KT Jr, McBride CM, Mera R. Microsatellite instability, prognosis and metastasis in gastric cancers from a low-risk population. Int J Cancer 89:444-452, 2000
34) Chiaravalli AM, Cornaggia M, Furlan D, Capella C, Fiocca R, Tagliabue G, Klersy C, Solcia E. The role of histological investigation in prognostic evaluation of advanced gastric cancer: analysis of histological structure and molecular changes compared with invasive pattern and stage. Virchows Arch 439:189-199, 2001
35) Wu MS, Lee CW, Sheu JC, Shun CT, Wang HP, Hong RL, Lee WJ, Lin JT. Alterations of BAT-26 identify a subset of gastric cancer with distinct clinicopathologic features and better postoperative prognosis. Hepatogastroenterology 49:285-289, 2002
36) Wirtz HC, Muller W, Noguchi T, Scheven M, Ruschoff J, Hommel G, Gabbert HE. Prognostic value and clinicopathological profile of microsatellite instability in gastric cancer. Clin Cancer Res 4:1749-1754, 1998
37) Marcus VA, Madlensky L, Gryfe R, Kim H, So K, Miliar A, Temple LK, Hsieh E, Hruki T, Narod S, Bapat BV, Gallinger S, Redston M. Immunohistochemistry for hMLH1 and hMSH2: a practical test for DNA mismatch repair-deficient tumors. Am J Surg Pathol 23:1248-1255, 1999