

Microsatellite Instability Is Associated With a Better Prognosis for Gastric Cancer Patients After Curative Surgery

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Abstract

Background Microsatellite instability (MSI) is one of the leading mechanisms for the carcinogenesis of gastric cancer. Its prognostic value is controversial.

Methods Between May 1988 and Oct 2003, a total of 214 gastric cancer patients undergoing curative surgery were enrolled, and their MSI statuses were classified as MSI-H (high) or MSI-L/S (low/stable). The clinicopathologic characteristics of MSI-H and MSI-L/S gastric cancers were compared.

Results The MSI-H tumors accounted for 11.7 % ($n = 25$) of the 214 total gastric cancers. Although not statistically significant, the MSI-H gastric cancers were more frequently located in the lower third of the stomach (64 % vs. 49.2 %) and were more often the intestinal type (72 % vs. 61.4 %)

compared to the MSI-L/S gastric cancers. The MSI-H gastric cancers had a significantly better 5-year overall survival (OS) rate (68 % vs. 47.6 %, $p = 0.030$) and a trend of a better 3-year disease-free survival rate (71.8 % vs. 55.2 %, $p = 0.076$) compared to the MSI-L/S gastric cancers. A multivariate analysis revealed that pathologic TNM stage and MSI status were the independent prognostic factors for OS after curative surgery.

Conclusions Compared to MSI-L/S tumors, MSI-H tumors are associated with a better OS rate for gastric cancer patients after R0 resection.

Introduction

Although the worldwide incidence of gastric cancer has been declining, it remains the fourth most common cancer

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and the second most common cause of cancer deaths [1]. Surgical resection and lymphadenectomy are the mainstays for curing gastric cancer, and the tumor stage provides an important prognostic prediction. Similar to colorectal cancer, there are two major pathways involved in the pathogenesis of gastric cancer: the chromosomal instability pathway and the microsatellite instability (MSI) pathway [2–5]. Somatic alterations demonstrate a DNA mismatch-repair (MMR) deficiency. Microsatellites are DNA regions containing short tandem repeats of one to six nucleotide motifs that frequently occur in the human genome. MSI is a common feature of gastric cancers and reflects an underlying MMR deficiency in the tumor, which is most commonly caused by the MMR genes (*hMLH1*, *hMSH2*). Alternatively, MSI is caused by the inactivation of *hMLH1* (but not *hMSH2*) via epigenetic promoter methylation. The incidence of MSI in gastric cancer has been reported to be 8 % to 25 % [6–12].

The phenotype of MSI stomach cancer is associated with the following characteristics: (1) it is almost always of the intestinal, rather than the diffuse Lauren's type of stomach cancer, which is much more frequently located in the distal (antrum) regions of the stomach instead of the proximal (body and cardia) regions; (2) it tends to be large and expansive; (3) it seldom gives rise to lymph node metastases [3, 13–18].

With respect to gastric cancer, some studies have reported that MSI is associated with a better survival [7, 8, 19–22], and other studies have found no significant differences between MSI and MSS [12, 14, 20, 23, 24]. Whether MSI can serve as a useful biomarker for survival prediction in gastric cancer is still unknown.

The aim of this study was to investigate the incidence of MSI-H tumors in Taiwanese gastric cancer patients and to clarify their clinicopathologic features and prognostic value.

Materials and methods

Between May 1988 and Oct 2003, gastric cancer patients who underwent curative resection were enrolled in this study. The institutional review board at the Taipei Veterans General Hospital approved this study, and written informed consent for tissue collection was obtained from all of the patients. Any patients with a history of gastric surgery or a pathologic diagnosis other than adenocarcinoma were excluded from the study. A total of 214 gastric cancer patients were enrolled in the study.

The pathologic staging of the gastric cancer was assessed after the surgery according to the seventh American Joint Committee on Cancer/Union Internationale Contre le Cancer (AJCC/UICC) TNM classification [25]. Specialized

gastric cancer surgeons performed all of the operations. The data were prospectively collected and recorded in a computer, and the patients' follow-up conditions were regularly updated.

Before surgery, all of the patients underwent chest radiography, abdominal sonography, or computer tomography (CT) for tumor staging. The patients were evaluated on the basis of their sex, age, tumor size, tumor location, operative methods, combined organ resection, pathologic tumor and lymph node stage, lymphovascular invasion, stromal reaction type, gross appearance, and recurrence pattern. For the stromal reactions, a cancer with a small number of stromal cells was classified as medullary, and a cancer with a large number of stromal cells was classified as scirrhous. An intermediate cancer was histologically intermediate (between the medullary and scirrhous types). The gross appearance of a tumor was classified as well defined (superficial or Bormann types I and II) or ill-defined (Bormann types III and IV).

A total or distal subtotal gastrectomy was performed depending on the distance between the cardia and the tumor. A margin of 3 cm was needed for superficial and well-defined tumors; a margin of 5 cm was needed for advanced or poorly defined tumors. A subtotal gastrectomy is the standard procedure for distal gastric cancer, whereas a total gastrectomy is the more common procedure for proximal gastric cancer. For early gastric cancer, at minimum a D1 + α dissection was performed. For advanced gastric cancer patients, the minimum of a D2 lymph node dissection was performed, except in those for whom curative resection was not possible. For D2 resection, combined-organ (i.e., en bloc) resection—including hemipancreaticosplenectomy, splenectomy alone, partial liver resection, and transverse colectomy—was sometimes performed to facilitate curative resection.

Follow-up

Overall survival was calculated from the time of surgery until death or the last follow-up contact. None of the patients received preoperative chemotherapy. Before 2008, adjuvant chemotherapy or radiotherapy after curative surgery was not routinely performed. Rather, it was performed only when tumor recurrence was diagnosed or highly suspected. Since 2008, adjuvant therapy (e.g., TS-1) was prescribed for stage II or stage III patients in our hospital after curative surgery because of its proven survival benefit [26].

Follow-up assessments were performed every 3 months for the first 5 years after surgery and every 6 months thereafter until the patient's death. The follow-up procedures included medical histories, physical examinations, routine blood tests, liver function tests, measurements of

the patients' tumor marker levels—carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9—chest radiographs, and other imaging studies. All routine procedures were performed by a surgeon; the upper endoscopies were performed by a gastroenterologist; and the upper gastrointestinal series, abdominal sonography, and CT scans were performed by a radiologist.

Biopsy sampling confirmed recurrent disease or distant metastases. We did not perform biopsies of new, multiple pulmonary lesions or lesions characteristic of osseous metastases that were noted during CT or whole-body bone scans. A tumor recurrence in the hepatoduodenal ligament, celiac axis, or peripancreatic region was considered a locoregional recurrence. We defined remote lymphatic metastasis (para-aortic, Virchow's, and inguinal nodes) and pulmonary lymphangitic spread as distant lymphatic recurrences. Using metallic staples at both the proximal and distal cut ends, we were able to readily identify the anastomotic sites and therefore could diagnose a recurrence at the anastomotic sites or duodenal stumps. Recurrence was classified as locoregional, hematogenous, distant lymphatic, or peritoneal. Patients who had recurrence of gastric cancer after surgery underwent chemotherapy with cisplatin 20 mg/m², fluorouracil 450 mg/m², and leucovorin 90 mg/m², given simultaneously in 0.5 liter of normal saline. It is infused intravenously over 96 h every 21 days. This treatment was delayed or modified according to the protocol if the patient displayed toxic effects.

Microsatellite instability analysis

Normal and tumor DNA was extracted from the formalin-fixed, paraffin-embedded (FFPE) tissues or fresh frozen tissues stored at -80 °C or in liquid nitrogen. After DNA was purified using the QIAamp Tissue Kit (Qiagen GmbH, Hilden, Germany), the quantitative DNA analysis was performed by measuring the optical density (OD) at a wavelength of 260 nm and 280 nm. The DNA quality was confirmed by the OD ratio of 260/280.

The purified DNA was amplified using the fluorescent polymerase chain reaction (PCR). According to the international criteria for the determination of MSI [27], five reference microsatellite markers were used: D5S345, D2S123, BAT25, BAT26, D17S250. The primer sequences were obtained from GenBank (www.gdb.org). The PCR products were denatured and analyzed via electrophoresis on 5 % denaturing polyacrylamide gels, and the results were analyzed using an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA). The presence of novel alleles that were observed in the PCR products from the tumor DNA but not observed in PCR products from the corresponding normal DNA were scored as MSI at that particular locus, as reported in a previous study [28].

Any samples with two or more loci of instability of five markers were classified as MSI-H, and any samples with one MSI or no evidence for MSI were classified as MSI-L/S. The analysis was repeated if the results were equivocal.

Statistical analysis

The statistical analyses were performed using SPSS software (version 16.0 for Windows; SPSS, Chicago, IL, USA). All of the results in the text and tables are presented as means ± standard deviations (SD). The categorical data were compared using a χ^2 test with a Yates correction or Fisher's exact test. Multivariate logistic regression analysis was conducted to determine the differences between the two groups. The overall survival (OS) was measured from the operation date to the date of death or the final follow-up. The disease-free survival (DFS) was defined as the length of time after surgery for gastric cancer during which a patient survives without tumor recurrence. The distributions of OS and DFS were estimated using the Kaplan–Meier method. Cox proportional hazards models were used to explore the association of clinical parameters with OS and DFS. A *p* value of <0.05 was considered statistically significant.

Results

The mean age of all of the gastric cancer patients was 66.8 ± 12.4 years. The mean follow-up duration was 25.5 months (range 1–243 months). Of the 214 patients, 152 (71 %) were men, and 62 (29 %) were women. Of all of the gastric cancers, 28 were located in the upper third of the stomach (13.1 %), 68 in the middle third of the stomach (31.8 %), 109 in the lower third of the stomach (50.9 %), and 9 in the entire stomach (4.2 %). The cancer stage distribution was as follows: 68 (24.4 %) stage I, 71 (25.4 %) stage II, and 140 (50.2 %) stage III. MSI-H and MSI-L/S gastric cancers accounted for 11.7 % (*n* = 25) and 88.3 % (*n* = 189) of the patients, respectively. The clinicopathologic characteristics of these patients are summarized in Table 1. The MSI-H gastric cancers were more frequently associated with lower-third gastric tumors (64 % vs. 49.2 %, *p* = 0.419); and they were more strongly associated with intestinal-type cancers than were the MSI-L/S gastric cancers (72 % vs. 61.4 %, *p* = 0.381). However, the clinicopathologic characteristics were not significantly different between MSI-H and MSI-L/S tumors.

Disease-free survival

The MSI phenotype was not associated with DFS at each stage in our gastric cancer patients. However, MSI-H

Table 1 Clinicopathologic profiles of all of the gastric cancer patients after curative surgery

Parameter	All patients (<i>n</i> = 214)	MSI-H (<i>n</i> = 25)	MSI-L/S (<i>n</i> = 189)	<i>p</i>
Age (years)	66.8 ± 12.4	64.8 ± 10.1	67.1 ± 12.7	0.377
Sex (M/F)	152/62	18/7	134/55	1.000
Tumor location				
Upper third of the stomach	28 (13.1 %)	2 (8 %)	26 (13.8 %)	
Middle third of the stomach	68 (31.8 %)	7 (28 %)	61 (32.3 %)	
Lower third of the stomach	109 (50.9 %)	16 (64 %)	93 (49.2 %)	
Whole stomach	9 (4.2 %)	0	9 (4.8 %)	0.419
Tumor size (cm)	5.9 ± 2.7	5.0 ± 1.9	6.0 ± 2.8	0.083
Lauren's classification				
Intestinal type	134 (62.6 %)	18 (72 %)	116 (61.4 %)	
Diffuse type	80 (37.4 %)	7 (28 %)	73 (38.6 %)	0.381
Stromal reaction type				
Medullary	42 (19.6 %)	6 (24 %)	36 (19 %)	
Intermediate	109 (50.9 %)	13 (52 %)	96 (50.8 %)	
Scirrhus	63 (29.4 %)	6 (24 %)	57 (30.2 %)	0.753
Lymphovascular invasion				
Absent	58 (27.1 %)	8 (32 %)	50 (26.5 %)	
Present	156 (72.9 %)	17 (68 %)	139 (73.5 %)	0.633
Gross appearance				
Well defined	90 (42.1 %)	13 (52 %)	77 (40.7 %)	
Ill-defined	124 (57.9 %)	12 (48 %)	112 (59.3 %)	0.291
Pathologic T category (T1/T2/T3/T4)	44/29/60/81	6/6/8/5	38/23/52/76	0.173
Pathologic N category (N0/N1/N2/N3)	75/35/33/71	8/6/7/4	67/29/26/67	0.089
Pathologic TNM stage				
I	68 (24.4 %)	6 (22.2 %)	62 (24.6 %)	
II	71 (25.4 %)	12 (44.4 %)	59 (23.4 %)	
III	140 (50.2 %)	9 (33.3 %)	131 (52 %)	0.151

Results are given as the mean ± SD or the number and percent

MSI-H: microsatellite instability-high; MSI: microsatellite instability-low/stable

tumors were associated with a trend of a better 3-year DFS compared to the MSI-L/S tumors in all patients who underwent curative resection (71.8 % vs. 55.2 %, $p = 0.076$).

We used Kaplan–Meier survival analysis to assess six prognostic factors: age, sex, tumor size, Lauren's classification, pathologic TNM stage, and MSI status. Univariate analysis showed that the tumor size and pathologic TNM stage were significantly associated with DFS (Table 2). The two variables were included in a multivariate Cox proportional hazards model with forward logistics regression stepwise procedure to adjust for the effects of covariates (Table 3). In this model, we demonstrated that only pathologic TNM stage was an independent prognostic factor for DFS.

Overall survival

There is a trend of better 5-year OS rates for MSI-H tumors than for MSI-L/S tumors at each TNM stage. We found that although all patients were enrolled the 5-year OS rate

was significantly higher for patients with MSI-H tumors than for those with MSI-L/S tumors (68 % vs. 47.6 %, $p = 0.030$).

We used Kaplan–Meier survival analysis to assess six prognostic factors: age, sex, tumor size, Lauren's classification, pathologic TNM stage, and MSI status. Univariate analysis showed that the following five factors were associated with overall survival: age, sex, tumor size, pathologic TNM stage, and MSI status (Table 2). All five variables were included in a multivariate Cox proportional hazards model using the forward logistics regression stepwise procedure to adjust for the effects of covariates (Table 3). In that model, we demonstrated that pathologic TNM stage and MSI status were independent prognostic factors for OS.

Discussion

To our knowledge, this hospital-based study investigating the MSI status of gastric cancer patients after curative

Table 2 Univariate analysis of factors affecting overall survival of gastric cancer patients after curative surgery by the Kaplan–Meier method

Factor	Disease-free survival			Overall survival		
	HR	95 % CI	<i>p</i>	HR	95 % CI	<i>p</i>
Age (years)						
<65	1.00			1.00		
≥65	1.37	0.909–2.069	0.132	1.56	1.068–2.290	0.022
Sex						
Male	1.00			1.00		
Female	0.77	0.509–1.169	0.221	0.61	0.408–0.902	0.013
Tumor size (cm)						
<5	1.00			1.00		
≥5	2.45	1.593–3.765	<0.001	2.08	1.432–3.009	<0.001
Lauren's classification						
Intestinal type	1.00			1.00		
Diffuse type	1.36	0.932–1.981	0.111	1.16	0.818–1.632	0.413
Pathologic TNM stage						
I	1.00			1.00		
II	2.97	1.378–6.384		1.43	0.803–2.555	
III	8.55	4.267–17.14	<0.001	4.53	2.768–7.405	<0.001
MSI status						
MSI-L/S	1.00			1.00		
MSI-H	0.57	0.312–1.021	0.076	0.51	0.273–0.937	0.030

HR hazard ratio, CI confidence interval

Table 3 Multivariate Cox proportional-hazards model using the forward logistics regression stepwise procedure for analysis of the survival of the gastric cancer patients after curative surgery

Parameter	Disease-free survival			Overall survival		
	HR	95 % CI	<i>p</i>	HR	95 % CI	<i>p</i>
Pathologic TNM stage						
I	1.00			1.00		
II	2.97	1.378–6.384		1.32	0.734–2.372	
III	8.55	4.267–17.14	<0.001	4.39	2.677–7.195	<0.001
MSI status						
MSI-L/S				1.00		
MSI-H				0.51	0.273–0.943	0.032

surgery is one of the largest series from a single institution. Our results showed that MSI-H tumors account for 11.7 % of gastric cancers. The reported incidence of MSI in gastric cancer varies by 8 % to 25 % [6–12]. The range of the incidence is primarily due to selection bias and varying definitions of MSI-H (i.e., the choice of microsatellite markers and the threshold for MSI-H tumors). As shown in Table 4, the markers for MSI testing ranged from 2 [7] to 11 [21] mononucleotide microsatellite markers. In an attempt to overcome this confusion, the National Cancer Institute proposed a panel of five microsatellite markers for the uniform analysis of MSI [28]. This panel included three

dinucleotide (D5S346, D2S123, D17S250) and two mononucleotide (BAT26, BAT25) repeats.

Similar to previous reports [3, 13–18] this study demonstrated that MSI-H tumors are frequently associated with a distal stomach tumor location and an intestinal Lauren's type of stomach cancer.

To clarify the role of MSI in gastric cancer, studies that were published through August 31, 2011 were searched and identified using the electronic PubMed database (<http://www.pubmed.com>). The key words included “gastric cancer,” “prognosis,” and “MSI.” The characteristics of the eligible studies are summarized in Table 4. In the

Table 4 Summary of the studies examining the gastric cancer patients' MSI statuses and the correlation between MSI and survival

Study	No. of patients	No. of markers analyzed	Threshold for MSI-H	% of MSI-H	DFS <i>p</i>	OS <i>p</i>
Beghelli [7]*	463	2	≥1 marker	16.0		0.005**
Corsol [8]	250	5	≥2 markers	25.2		0.002
An [12] ^a	1990	5	≥2 markers	8.5	>0.05	>0.05
Ottini [14]	108	6	≥2 markers	30.6		0.88
De Manzoni [19]	44	9	≥5 markers	18.2		0.029**
Gazvoda [20] ^b	73	6	>29 %	12.0		0.022
Hayden [21]	101	11	≥1 locus	21.0		0.15
Schneider [22]	169	8	≥ 30 %	18.2		0.027
An [23]	81	5	≥ 2 markers	19.0	>0.1	0.15
Perez [24] ^c	24	5	≥1 marker	20.8		>0.1
Fang (present study)	214	5	≥2 markers	11.7	0.076	0.032**

DFS disease-free survival, OS overall survival

^a No significant differences in the DFS rates of the MSS/MSI-L and MSI-H patients in stage I, II, III, or IV

^b MSI-H mean survival time: 4.2 years; MSI-L mean survival time: 1.7 years; *p* = 0.022

^c A difference between the mean OS and the DFS was observed only in the stage III patient subgroup

* The 5-year survival rates revealed significant differences only between the MSS and MSI neoplasms for stage II cancers (HR = 0.3; 95 % CI 0.1–0.8; *p* = 0.011)

** Multivariate results

literature review, for which 10 studies were included, the present study and two other studies [7, 19] performed multivariate analyses of OS; they all revealed a significant role for MSI, resulting in better survival rates. Only An et al. [12] and Perez et al. [24] analyzed DFS in gastric cancer with R0 resection. Their results indicated no significant differences between the MSI-H and MSI-L/S tumors in terms of DFS. Interestingly, although not statistically significant, our data showed a trend of better OS rates for patients with MSI-H tumors than for those with MSI-L/S tumors at each stage. However, the difference in OS between MSI-H and MSI-L/S gastric cancer was significant only when all patients were enrolled. Furthermore, our study showed a trend of better DFS for patients with MSI-H tumors than for those with MSI-L/S tumors after curative resection. This may be explained by the small number of MSI-H tumors in our series, which did not provide sufficient statistical power to achieve a statistically significant difference. The chief limitation of this study is that few MSI-H gastric cancer cases were included, which rendered the subgroup analyses difficult. A larger sample size may be needed to clarify the actual role of MSI-H in DFS in gastric cancer patients.

MSI-H colon cancer is frequently located in the right-sided colon and is associated with a mucinous histology [29, 30]. The inactivating mutations in MSI-H colon cancers cluster to similar hypermutable repetitive DNA sequences present in the coding regions of genes important to cell growth and cell survival, such as transforming growth factor- β receptor type II (TGF β -RII), insulin-like

growth factor receptor type II (IGF-RII), transcription factor E2F-4, Bcl-2 protein (BAX), caspase-5, and methyl-CpG binding domain protein 4 (MBD4) [31–36]. MSI-H gastric cancer is associated with antral tumor location and intestinal-type differentiation. Similar to colon cancer, some genes with simple, tandem repeat sequences within their coding regions have been found to be specifically altered in gastric cancers displaying MSI, including BAX, hMSH3, hMSH6, E2F-4, TGF β -RII, and IGF-RII, which are known to be involved in the regulation of cell-cycle progression and apoptotic signaling [37, 38]. Moreover, similar to colorectal cancer, the presence of methylation of the *hMLH1* gene is strongly associated with loss of hMLH1 protein expression and the MSI-H phenotype in gastric cancer [39–41]. Hypermethylation of CpG islands in the promoter region of the *hMLH1* gene is associated with decreased hMLH1 protein expression and often occurs in MSI-H gastric cancer, indicating that epigenetic inactivation of this gene in association with promoter methylation may underlie MSI [40, 41]. Consequently, we will make an effort to investigate the molecular profile of MSI-H gastric cancer in our future work. The limitation of this study is that the biological markers are not validated internally with a validation set. External validation is also needed.

An et al. [12] reported that patients with MSS/MSI-L gastric cancers obtained potential DFS benefits from 5-fluorouracil (5-FU)-based adjuvant chemotherapy. By contrast, 5-FU-based adjuvant chemotherapy did not provide DFS benefits to stage II and III gastric cancers with MSI-H. Because adjuvant chemotherapy (e.g., TS-1) was

initiated in our hospital only in 2008, our patients did not receive routine adjuvant chemotherapy before 2008. We could not compare the effect of chemotherapy on MSI-H versus MSI-L/S patients in this study.

Conclusions

Based on our hospital-based study, MSI-H gastric cancers were associated with significantly better OS and a trend of better DFS compared to MSI-L/S gastric cancers after curative surgery. A multivariate analysis revealed that sex, pathologic TNM stage, and MSI status are independent prognostic factors for OS.

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Conflict of interest None.

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