Microsatellite Instability in Japanese vs European American Patients With Gastric Cancer

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Background: The stage-stratified survival following gastrectomy for gastric cancer is far better in Japan than in the United States. The process of carcinogenesis may differ in gastric cancers from Japan and the United States, accounting for prognostic differences, as patients of Asian descent treated in United States also exhibit superior survival in comparison with non-Asian patients.

Hypothesis: The phenotype of gastric cancer differs between Japanese and American patients.

Design: Retrospective case-case (blinded) study.

Setting: University hospitals in Japan and the United States.

Patients and Methods: We compared the frequency of microsatellite instability (MSI) at 7 loci from formalin-fixed paraffin-embedded gastrectomy specimens, between cases of gastric cancer at Hitachi General Hospital (N=18) and in US patients of European descent treated in Orange County, Calif (N=20). Microsatellite instability, Lauren classification, and T stage were determined without knowledge of the country of origin of the specimens.

Main Outcome Measure: The frequency of MSI in Japanese vs European American gastric cancer specimens.

Results: The frequency of MSI in Japanese gastric carcinoma specimens was higher than in specimens from American patients of European descent (39% vs 20%, respectively). In contrast, a high frequency of MSI was demonstrated in only 3 European American specimens (15% of all specimens in this group). Tumors from Japanese and American men were more likely to demonstrate MSI than those from women (50% vs 5.6%, respectively; P=.004). Among advanced-stage tumors, Japanese specimens were significantly more likely to demonstrate MSI (55%) than European American specimens (7.1%; P=.02). Specimens from Japan and America demonstrating MSI were equally likely to be from men, involve the gastroesophageal junction, and demonstrate intestinal histologic abnormalities.

Conclusions: Advanced gastric cancers from Japan are more likely to demonstrate MSI. These data warrant a study of larger numbers of patients to assess whether differences in MSI expression correlates with prognostic differences between gastric carcinoma in patients in Japan vs the United States.

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The affiliations of the authors appear in the acknowledgment section at the end of the article.
PATIENTS AND METHODS

Formalin-fixed paraffin-embedded gastrectomy specimens from Japan were obtained from consecutive patients with gastric tumors containing adequate amounts of cancerous and normal tissue to allow microdissection. They were provided by Minoru Okumura, MD (Hitachi General Hospital, Ibaraki, Japan). Formalin-fixed paraffin-embedded tissue gastrectomy specimens from Americans of European descent treated in Orange County, California, were obtained from consecutive cases of gastric cancer containing adequate amounts of cancerous and normal tissue to allow microdissection. The patient, or a family member in cases of deceased patients, provided informed consent. Demographic and clinical data were obtained from medical records or tumor registries. Tumors were graded according to Lauren criteria and staged by depth of invasion according to criteria of the International Union Against Cancer by an experienced surgical pathologist (P.C.) who was unaware of the country of origin of the specimen. DNA from cancerous and corresponding noncancerous tissues was isolated from 10-µm formalin-fixed, paraffin-embedded tissue sections by microdissection after delineation by a pathologist (F.L.). Tumors included in the study could be manually microdissected such that preparations included at least 70% tumor nuclei. DNA was extracted from 2 slides using the QiAamp DNA miniprep kit (Qiagen, Valencia, Calif), according to the manufacturer’s instructions. Approximately 12 µg of DNA were obtained from 2 slides.

Seven loci containing mononucleotide or dinucleotide repeat sequences were amplified: D5S346, BAT 25, BAT26, BAT 40, D2S123, D4S531, and D18S69. The sequences of primers used to amplify locus D2S123 were modified according to the methods of Umetani et al.49 Labeled primers were purchased from BioServe Technologies (College Park, Md). The polymerase chain reaction (PCR) method was performed in a total volume of 25 µL using 40nM specific primers, 20 to 30 ng of DNA, 3mM each deoxynucleotide, 0.15 U of Taq DNA polymerase (Roche, Mannheim, Germany), and 2.5 µL of 100mM Tris-HCl (pH=8.3) containing 15mM magnesium chloride and 500mM potassium chloride. Amplification was done by denaturing at 95°C for 2 minutes, followed by 11 cycles at 95°C for 30 seconds, 65°C for 1 minute and 72°C for 1 minute. This was followed by 26 cycles at 95°C for 30 seconds, 55°C for 1 minute, 72°C for 1 minute, 60°C for 2 minutes; and 72°C for 10 minutes on a PTC-100 PCR thermal cycler (MJ Research Inc, Watertown, Mass). Amplification of 97% of loci was accomplished. Aliquots of each amplified product were mixed with 2 µL of 100% formamide, 4 µL of running buffer (25mM EDTA, 50 mg/mL of blue dextran), and 8nM Rox size standards (Perkin-Elmer, Foster City, Calif). Samples were heated at 95°C for 2 minutes prior to gel electrophoresis using an ABI Prism 377 DNA Sequencer (Perkin-Elmer). Analysis was done using GeneScan 3.1.2 software (Perkin-Elmer). Microsatellite instability was assessed by comparison of the peak motif between tumor and normal tissue. Each microsatellite locus was reviewed and classified as MSI positive or microsatellite stable (MSS) by 2 observers who were unaware of the country of origin of the specimen (B.S.C. and D.J.P.). Microsatellite instability was defined as the presence of novel bands present in the PCR products obtained from tumor specimens that were not present in PCR products from corresponding normal tissue. A tumor was considered to exhibit a high frequency of MSI if 3 or more loci exhibited unequivocal instability, or a low frequency of MSI (MSI-L) if only a single locus demonstrated instability.

The study was approved by the internal review board of the University of California, Irvine.

The Fisher exact probability test (when comparisons involved values ≤ 5) or the χ2 tests were used to compare categorical variables, and the t test was used to compare continuous variables using SAS statistical software.20,31 P<.05 (2-tailed) was considered statistically significant.

RESULTS

DEMOGRAPHIC AND CLINICAL COMPARISON

Thirty-eight gastric tumors treated by gastrectomy were studied, including 18 specimens from Japan and 20 from Americans of European descent. Japanese patients were slightly younger than European American patients (65 years vs 73 years, respectively), though this difference was not statistically significant. There were no statistical differences in sex, Lauren classification, T stage, or tumor site (gastroesophageal junction vs nongastroesophageal junction) between Japanese and European American patients (Table 1).

JAPANESE AND EUROPEAN AMERICAN SPECIMENS COMBINED

Eleven (29%) of 38 gastric carcinomas demonstrated MSI at 1 or more of the 7 microsatellite loci examined. The

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Microsatellite instability was demonstrated in 20% of gastric cancer specimens from American patients with MSI. The mean age of patients whose tumors demonstrated MSI (66 years) was similar to that of tumors that demonstrated MSS (70 years; Table 2). Tumors from men were more likely to demonstrate MSI than those from women. Specimens from 10 of 20 men demonstrated MSI, in comparison with specimens from 1 of 18 women (P = .003). Stage of disease was classified as either early (T1) or advanced (T2 or T3). Microsatellite instability was not associated with stage, Lauren classification, or tumor site (gastroesophageal junction vs nongastroesophageal junction).

SPECS FROM AMERICAN PATIENTS OF EUROPEAN DESCENT

Microsatellite instability was demonstrated in 20% of gastric cancer specimens from Americans of European descent. Microsatellite instability was demonstrated at 3 or more loci in 3 cases, and at a single locus in the other case of MSI. The mean age of American patients who demonstrated MSI was similar to that of American patients with MSS tumors. American men were more likely to exhibit MSI than women: all 11 women in the cohort were MSI-negative, while only 3 of 16 American men demonstrated MSI in contrast with MSS tumors. American men were more likely to exhibit MSI than women: all 11 women in the cohort were MSI-negative, while only 3 of 16 American men demonstrated MSI, though this was not statistically significant (P > .10; Table 3).

Although there is a nonsignificant difference in the rate of MSI between American and Japanese patients, the frequency of MSI-positive specimens in Japanese patients was twice the frequency in American patients. Only 3 of the 16 American men demonstrated MSI compared to 11 of 18 Japanese men (P = .08), indicating that Japanese men are twice as likely to demonstrate MSI. There was no statistically significant difference in the frequency of MSI-positive specimens between American and Japanese women (P = .10). This difference was not statistically significant. In fact, we would have needed to analyze 99 cases from each region to detect a statistically significant difference between the frequencies of MSI.

### SPECIMENS FROM JAPANESE PATIENTS

Microsatellite instability was demonstrated in 39% of Japanese gastric cancer cases. One specimen demonstrated MSI at 2 loci, and the remaining MSI-positive specimens demonstrated MSI at a single locus. The mean age of the Japanese patients who demonstrated MSI (61 years) was lower than that of Japanese patients who did not demonstrate MSI (67.8 years), though this was not statistically significant (P > .10; Table 4). Japanese men were more likely to demonstrate MSI than Japanese women. Microsatellite instability was demonstrated in 55% of male and 14% of female patients (P = .08). Japanese specimens that demonstrated MSI were more likely to be of advanced stage (86% were T2 or T3 lesions) than MSS specimens (45% were T2 or T3 lesions; P > .10).

### COMPARISON OF JAPANESE AND EUROPEAN AMERICAN SPECIMENS

The rate of MSI in Japanese gastric carcinoma specimens was higher than in specimens from American patients (39% vs 20%, respectively). This difference was not statistically significant. In fact, we would have needed to analyze 99 cases from each region to detect a statistically significant difference between the frequencies of MSI.
we demonstrated, assuming a 2-tailed $\alpha$ level of .05 and a $\beta$ level of .20. In contrast, MSI was demonstrated at 3 or more loci in 15% of American patients and in none of the Japanese patients. The average age of Japanese patients with MSI-positive gastric cancer specimens (61.1 years) was younger than the average age of American patients with MSI-positive specimens (75.3 years). Microsatellite instability–positive specimens were more likely to be of advanced stage (T2 or T3) among Japanese patients (86%), and of early stage (75%) among Americans. Among advanced gastric cancer cases (T2 or T3 lesions), Japanese specimens were significantly more likely to demonstrate MSI (55%) than European American specimens (7.1%; $P = .02$). There were no statistical differences in sex, Lauren histology, and tumor site between Japanese and European American MSI-positive specimens (Tables 3 and 4).

**COMMENT**

There is no clear explanation for the marked difference in gastric cancer survival between patients in Japan and the United States. Differences in surgical technique and pathologic criteria for malignancy have been thought to account for these differences. $^{1-5}$ Recent data from cancer registries and an individual cancer center in America, however, indicate that Asian patients with gastric cancer treated in America also have superior adjusted survival, as well as a decreased propensity for lymph node metastases. $^{6-9}$ These data indicate that gastric cancer in Asians may differ biologically from gastric cancer in European Americans. Few studies have compared the molecular biology of gastric cancers from the 2 regions. This study represents the first investigation to compare the frequency of MSI between European American and Japanese gastric cancer specimens.

We found differences in the expression of MSI by Japanese and European American gastric cancer specimens. Microsatellite instability was demonstrated in a higher proportion of Japanese than American cases (39% vs 20%, respectively), although an MSI-H, defined as instability of greater than 30% of amplified loci, was detected among only American patients. Advanced gastric cancers from Japan were significantly more likely to demonstrate MSI than European American specimens. This difference in MSI expression between Japanese and European American patients is consistent with the hypothesis that the process of carcinogenesis differs in gastric cancers from the 2 regions.

Two other studies have compared rates of MSI between patients from Asia and North America. Sepulveda et al $^{30}$ found a significantly higher frequency of MSI in Korean gastric cancer specimens (59%) than in specimens from America (12%) or Colombia (35%). A high frequency of MSI was also detected more frequently in Koreans (50%) than in Americans (7%) or Colombians (15%). Tumor and normal tissue DNA used for amplification in the study were isolated from endoscopic biopsy specimens in the cases of Korean and Colombian patients, and from gastrectomies in American patients. These results were not duplicated in a study that compared the rate of MSI in specimens from 142 Americans (29%), 20 Koreans (30%), and 6 Chileans (33%) undergoing a gastrectomy. $^{30}$ Differences in the frequency of MSI between the 2 studies may reflect differences in primer pairs used for amplification. While both groups used 5 or more primer pairs, only 2 primer pairs were identical in the 2 studies.

Published rates of MSI in American, European, and Asian cancer specimens vary widely (range, 13%-67%) and reflect methodological differences such as quality and quantity of tissue, methods of DNA extraction, number and identity of primer sets used for amplification, definition of MSI, and methods of detection. Disparities between reported rates of MSI may also reflect the composition of populations chosen for study. In 1998, the National Cancer Institute (NCI) (Bethesda, Md) convened a consensus conference on MSI to develop international criteria for MSI and unify study in the field.

The strengths of the current investigation include (1) the amplification of loci recommended by the NCI consensus committee, (2) the use of automated technology to accurately assess MSI, and (3) the determination of MSI by investigators who were unaware of the country of origin of the specimens. Seven primer sets were analyzed to increase the sensitivity for detecting MSI at least one locus. Four of 5 of these primer sets amplify the reference panel loci recommended for the evaluation of MSI by the NCI. $^{52}$ The fifth NCI-recommended reference locus, D17S250, did not amplify reliably, possibly because of DNA damage resulting from fixation, as well as the age of the samples. Therefore, the alternate loci D18S69 and BAT 40 (recommended by NCI) were amplified. Finally, the D4S531 locus, which has been successfully amplified in population-based studies of hereditary nonpolyposis colorectal cancer, was amplified. $^{53}$

Chong et al $^{35}$ also found that MSI was demonstrated more often in Japanese cases of advanced gastric cancer compared with early gastric cancer (39% vs 1%). Subsequent studies indicate that high-frequency MSI is associated with favorable prognostic clinical and patho-

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*All data are presented as number (percentage) of patients unless otherwise indicated. MSI+ indicates microsatellite instability positive; MSS, microsatellite stable; and GEJ, gastroesophageal junction.

**Table 4. Demographic, Clinical, and Pathologic Features of Japanese Gastric Cancer Stratified by MSI Status**
logic factors. Studies of largely advanced gastric cancer cases from Japan, Korea, North America, Italy, Taiwan and Portugal correlated high-frequency MSI with the absence of lymph node metastases, intestinal histology (Lauren classification), expanding growth pattern (Ming classification), antral location, minimal desmoplasia, as well as superior unadjusted survival.26-28,30-32,36,39-44 Further studies indicate that high-frequency MSI is associated with specific genetic and epigenetic mutations.40,42,44 Studies of high-frequency MSI and prognostic variables, however, have been inconsistent. A large study of German gastric cancer patients found that high-frequency MSI was unassociated with specific clinical or pathologic variables or survival.38 Most studies have found that low-frequency MSI is associated with the unfavorable clinical and pathologic variables found in gastric cancers that do not demonstrate MSI.26-28,30-32,36 Studies from Portugal, however, indicate that low-frequency MSI is correlated with the absence of lymph node metastases and the presence of epigenetic mutations at rates nearly identical to those associated with high-frequency MSI.32,40 Further, both high-frequency MSI and low-frequency MSI were associated with prolonged unadjusted survival in a large study of North American patients with gastric cancer.30 We were unable to determine if differences in MSI expression correlated with prognostic differences between Japanese and European American gastric cancer cases. Further study to determine MSI in larger numbers of gastric cancer specimens from Japan and America, and to correlate MSI with prognostic data will be valuable.

Microsatellite instability was found in 50% of male patients with gastric cancer studied from both Japan and America, but it was rarely detected in female patients. An association between male sex and MSI has not been reported previously, even in studies with greater power than the current study to detect these associations. This finding may be attributed to the primers used for amplification, random chance, or a unique aspect of the populations studied. Prior study, however, indicates that sex influences gastric cancer phenotype. Signet ring histological findings are significantly associated with female sex by multivariate analysis.36 It is possible that the hormonal milieu of women influences the expression of MSI.

In conclusion, the present study was conducted on specimens obtained from Japanese and European American patients with gastric cancer, and MSI was determined using identical methods without knowledge of the country of origin. Gastric cancer tissue from Japanese patients demonstrated nearly twice the frequency of MSI, although high-frequency MSI was observed only in European Americans. Advanced tumors from Japan were statistically more likely to demonstrate MSI compared with tumors in European Americans. These data warrant a study of larger numbers of patients with gastric cancer to assess whether differences in MSI correlate with differences in prognosis, reflecting differences in the process of carcinogenesis between Japan and the United States.

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associated with favorable prognostic factors: histology of the intestinal type, antral location of the tumor, and node-negative status.

In the “Results,” the authors found definite differences between Japanese and American gastric cancers, with MSI being twice as common in the Japanese tumors. But as the rain in life and science all too often falls, MSI in the Japanese tumors was associated with advanced stage, whereas in the American tumors it was associated with early stage, the reverse of what one might a priori think. No correlation of MSI with the Lauren classification was observed. There was no correlation of MSI with location of the tumor. So life is a female dog with puppies and then you die.

My questions have to do with where the authors go from here. Dr Campbell points out that enlarging the study, enlarging the sample size considerably, and adding survival data would be very valuable. One of the questions of course, is whether the difference in MSI they observe in Japanese and American tumors is real and significant, or have they merely touched on biologic heterogeneity? And finally, a serious question: Do they abandon MSI altogether and look for a molecular phenotype that is more consistently correlated with TNM staging and/or survival (if such exists, and it hopefully does out there somewhere)?

Dr Butler: Dr O’Connell, Dr Wilson, members, and guests. As was stated in the introduction, it’s been clear for many years that the 5-year survival of gastric cancer in Japanese patients is far superior to that of American patients, even when stratified for stage. Part of that may be due to more accurate staging; part of it may also be due, at least in the early gastric cancers, to questions as to what the Japanese call invasive cancer, as opposed to how we define it here in the United States. But having said that, the Japanese firmly believe that their improvement is on the basis of their more extensive nodal dissection. When that has been tested in randomized trials (and there have been 4 major trials in Europe, Africa, and Asia) at least for Western patients, that turned out not to be the case. In point of fact, there was increased morbidity and mortality associated with the extended dissection. So the issue naturally presents itself, that this difference may be due to an altered biology.

Dr Theuer, who is the first author (and actually the senior author on this paper and who would be up here at the podium right now except for the fact that he doesn’t become a member until next year), in the year 2000, actually wrote 2 very important papers on gastric cancer. One was an epidemiologic study looking at 4000 gastric cancers in San Diego County, Orange County, and Imperial County, and what he showed was that survival, stratified by stage, was superior in the Asian patients. He also showed that Asian patients had a much higher prevalence of early gastric cancer.

A second paper that he wrote, which was probably even more important, looked at 10-year data from a university hospital—our hospital at UCI in Orange. While there were many factors that changed during those 10 years, including the economic viability of the hospital, the surgical approach to gastric cancer was the same through that 10-year period. The pathologic staging was also the same. Dr Theuer showed in our cohort of patients treated in the same fashion, the exact same thing, that even when you stratified for stage, survival was significantly superior in Asian patients as opposed to patients of European descent. So it suggests that there are changes in the biology, and that the natural history of gastric cancer is perhaps different in Asian patients than in Western patients.

As for the questions that Dr Goodnight raised, these are small numbers of patients, so whereas our correlates with MSI instability are not necessarily the same as have been found in other studies, there is a lot of work that still remains to be done in terms of which loci need to be looked at to determine MSI, and the methodology used to determine whether or not it is present. But what this study does show is that there are biological differences between the 2 groups. There have been some interesting data looking at intestinal metaplasia in patients with and without gastric cancer that also show that maybe MSI has a role to play in that, and maybe they can be used to identify patients for chemoprevention trials.

Finally, Dr Goodnight asked what other things might we look at. Clearly, as I think Dr Paz alluded to, the transcriptional profiling available with DNA chips that allows you to process a cancer and look for genetic changes in more than 30,000 genes is obviously a fruitful area for further investigation, and Dr Theuer’s laboratory is doing exactly that.