Application of the National Cancer Institute International Criteria for Determination of Microsatellite Instability in Ovarian Cancer

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Abstract

Recently, the National Cancer Institute (NCI) established criteria for determination of microsatellite instability (MSI) in colorectal tumors. Although the best panel of markers for ovarian tumors is not known, we evaluated epithelial ovarian cancers for MSI based on the NCI recommendations. One hundred and nine ovarian tumors were analyzed for MSI by gel analysis of paired germ-line and tumor DNA. PCR amplification and枝position of five microsatellite markers recommended by the NCI (BAT25, BAT26, D5S346, D2S123, and D17S250) and nine additional markers based on their genomic location (NME1, D10S197, D11S904, D13S175, DXS981, DXS6800, DXS6807, AR, and D3S1611). Tumors were characterized on the basis of high-discriminant MSI (MSI-H) if two or more of the five NCI markers showed instability or there was instability at 30% or more of all markers tested; or low-frequency MSI (MSI-L) if only one of the five NCI markers showed instability or <30% of all of the markers. All of the other tumors were considered microsatellite stable. On the basis of the NCI markers, 12 (11%) tumors demonstrated MSI-H, and 8 (7%) additional tumors had MSI-L. When all of the 14 markers were considered together, 13 (12%) tumors demonstrated MSI-H (based on 30% or more unstable loci), and 26 (24%) tumors had MSI-L. A single tumor identified to have MSI-H based upon all of the markers tested would have been classified as MSI-L based upon the NCI markers alone. Inclusion of an additional dinucleotide marker (NME1) to the NCI panel allowed detection of all of the tumors with MSI-H using only six markers. MSI-H occurs in ~12% of invasive ovarian tumors. For optimal detection of microsatellite instability in ovarian cancer, an additional marker (NME1) may be required, along with the five recommended by the NCI.

Introduction

Since the identification of the association of MSI\(^1\) with colorectal cancer in 1993 by three independent groups, it has been shown to play a significant role in several other cancers (1–3). MSI is identified when alleles of novel sizes are detected in microsatellite sequences derived from cancer DNA that are not present in normal tissues of the same individual. The discovery of MSI in colorectal cancers and its linkage to HNPCC in 1993 has resulted in many advances related to detection of cancer susceptibility, response to chemotherapy, and clinical outcome. Although the incidence of MSI with HNPCC is ~90%, it is also a distinctive feature in nearly 20% of sporadic colorectal tumors (3–6). MSI is also seen in a significant proportion of extracolonic tumors including breast, endometrial (7), gastric (8–10), and ovarian (11–14) cancer. HNPPC predisposition is associated with constitutional mutations in MMR genes hMSH2, hMLH1, hPMS1, hPMS2, hMSH3 (15), and hMSH6 (16). Cells deficient in MMR gene function are not able to correctly repair errors occurring during DNA replication. Because of their repetitive structure, which favors strand slippage, microsatellites are particularly prone to replication errors, and MSI is thus a hallmark of MMR deficiency.

Several different techniques have been used to detect MMR defects. These include in vitro assays, MSI, reverse transcription-PCR of MMR mRNA, and immunohistochemistry (17, 18). Each of these techniques has benefits and limitations. The most widely used technique involves using several microsatellite markers (19), but the optimal detection method for MMR defects is not known.

The incidence of reported MSI has been highly variable in various tumor models and is partly dependent on the type and number of markers used. A wide variety of markers ranging from mononucleotide to tetrانucleotide have been used. To standardize the markers used for microsatellite analysis, the NCI held a conference in 1997 to define uniform criteria for MSI (19). From this conference, five specific markers for microsatellite analysis in colorectal cancer were recommended (19). According to their recommendations, tumors may be characterized as having MSI-H if two or more of the five markers exhibit variations in microsatellite sequences or MSI-L if only one marker shows instability. Furthermore, MSS tissue is defined by microsatellite sequences of normal length at all of the five loci.

Although this panel has been validated in colorectal cancers, the optimal panel of markers for extracolonic cancers including ovarian cancer is not known. Thus, our goal was to evaluate the usefulness of the five markers that the NCI recommended for detection of MSI in ovarian cancer.

Materials and Methods

Preparation of Tissue. All of the samples were collected in compliance with requirements of the Institutional Review Board for the Protection of Human Subjects. Tumor samples were snap frozen. DNA isolation and preparation techniques have been reported previously (20, 21). Peripheral blood lymphocytes were obtained from patients as a source of normal DNA.

Genomic Instability Analysis. The five markers (BAT25, BAT26, D5S346, D2S123, and D17S250) recommended by the NCI were evaluated. Nine additional loci including dinucleotide (D3S1611, D10S197, D11S904, D13S175, and NME1), trinucleotide (androgen receptor), and tetrарucleotide (DXS981, DXS6800, and DXS6807) repeat sequences and representing different chromosomes were also evaluated. The rationale for selection of these markers has been reported previously (11). All of the markers were obtained from Research Genetics (Huntsville, AL). DNA derived from lymphocytes or tumor cells was amplified by PCR in 10-μl reaction mixtures containing 2 mm deoxynucleotide triphosphates, 1 unit of Taq polymerase, 1 μl of 10× buffer (Roche, Indianapolis, IN), 0.4–1.5 μl of 12.5 μM primer pairs (M13-tailed forward primer), and 0.5 μl of M13IRD700 (1 pmol; Licor, Lincoln, NE).

The abbreviations used are: MSI, microsatellite instability; HNPCC, hereditary nonpolyposis colorectal cancer; MMR, mismatch repair; NCI, National Cancer Institute; MSI-H, high-frequency MSI; MSI-L, low-frequency MSI; MSS, microsatellite stable.

References

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Reactions were carried out in a Biometra Trio-Thermoclock thermocycler (Biometra, Inc., Tampa, FL), beginning with a 95°C denaturing step for 5 min. Subsequently, each cycle began at 95°C for 45 s, with each cycle having a 72°C extension of 1 min. Three different annealing temperatures were used for the reaction: 68°C × 5 cycles (5 min each); 58°C × 5 cycles (2 min each); and 56°C × 25 cycles (2 min each). A final extension of 72°C for 10 min was performed. Normal and tumor DNA were placed side by side for each polymorphic marker. A patient was considered informative if two allelic bands were seen in normal DNA. MSI was identified when shifts were noted in the allelic bands in tumor DNA compared with normal DNA consistently in repeated experiments. All of the gels were reviewed by two of the authors independently (A. K. S. and R. H.). If there was discordance of opinion, then the gel was observed by a third investigator (R. E. B.).

Clinicopathological Variable Analysis. All of the patients underwent surgical exploration and cytoreduction as the initial treatment. Diagnosis was verified by pathology review at the institutional gynecological oncology tumor board. All of the patients were staged according to the International Federation of Gynecology and Obstetrics surgical staging system. The pathology for all of the patients was reviewed by a gynecological pathologist.

Statistical Analysis. χ² or Fisher’s exact test was used as appropriate to determine differences between variables using SPSS (SPSS, Inc., Chicago, IL). Kaplan-Meier survival plots were generated, and comparisons between survival curves were made with the log-rank statistic. P < 0.05 was considered statistically significant.

Results

DNA samples from 109 tumors and matched peripheral blood samples were analyzed using the five NCI designated markers as well as nine other microsatellite markers. Representative examples of microsatellite analysis are shown in Fig. 1. Tumors 323.01, 110.01, and 253.01 demonstrate MSI at three of the NCI designated loci shown, although no instability is noted in tumors 199.01, 97.01, and 323.01. The frequency of instability at each microsatellite locus is shown in Fig. 2. NME1 (13%) and D13S175 (13%) were the most frequently altered markers in the study cancers. Other frequently unstable loci included D3S1611 (12%), BAT25 (11%), and D5S346 (10%). DXS6807 displayed the least instability with only three tumors (2.7%) showing MSI.

The results of microsatellite analysis are presented in Table 1 in a matrix format. When we used the five NCI designated markers alone, 17 (11%) of the tumors demonstrated MSI-H and 8 (7%) additional tumors showed MSI-L. Eighty-nine tumors were microsatellite stable. When all of the markers were considered simultaneously, 15 (12%) of the tumors were considered MSI-H, and 26 (24%) of the tumors were considered MSI-L. The remaining 70 tumors were MSS. Among tumors with MSI-H based on all of the markers, the proportion of unstable loci ranged from 31 to 86% being altered. In those with MSI-L, the incidence of altered loci ranged from 7 to 21%. Overall, 39 (35%) tumors displayed some degree of instability.

Twelve of 13 tumors with MSI-H would have been detected by using the five NCI designated markers alone. However, 1 tumor with >30% of all loci with instability had only one of five unstable NCI markers. Inclusion of the NME1 marker would have allowed classification of this tumor as MSI-H. Thus, all of the tumors with MSI-H could have been detected using only six markers.

Some investigators have suggested previously that MSI can be characterized using mononucleotide markers without the need for...
microsatellite analysis has been used as the most common form of detection. The incidence of mutations in the DNA MMR genes may be useful alone or in conjunction with PCR-based MSI detection. The incidence of mutations in the DNA MMR genes appears to be lower in sporadic tumors with MSI-H (14, 45, 46). It is likely that other mechanisms, such as hypermethylation, may play a role in gene inactivation in these tumors (35, 47). Promoter methylation abnormalities in these genes may result in a MMR-deficient phenotype, which may affect clinical behavior and response to treatment (35). We are actively evaluating the role of promoter methyla-

### Discussion

Microsatellites are widely used for linkage analysis and studies on loss of heterozygosity and are generally highly polymorphic. Clinical definitions of syndromes, such as the HNPCC, are frequently unreliable, and molecular definitions are needed (19). Potential clinical uses of MSI may include diagnosis and determination of prognosis, prediction of response to treatment, and characterization of tumor biology (growth rate, progression of premalignant lesions, and metastasis; Ref. 19).

A variety of techniques have been used for identification of tumors with MMR defects ranging from PCR-based to immunohistochemistry (17, 25–28). We and others have attempted to use more global techniques for rapid and reproducible identification of genomic instability (11, 29). Although these techniques are sensitive for detecting MSI, they may be limited in being able to distinguish MSI-H from MSI-L. The current techniques for identifying MMR defects differ greatly, and the optimal technique remains unknown. PCR-based microsatellite analysis has been used as the most common form of analysis, because generally it is relatively simple to perform. However, different investigators have used a large variety of markers ranging from mononucleotide to tetranucleotide to perform this analysis. In addition, a precise definition of MSI has yet to emerge. To provide standardized criteria for the definition of MSI, the NCI held a consensus conference in 1997 and recommended five markers for detection of MSI in colon cancer (19). However, the optimal group of markers for MSI detection in ovarian cancer is not known.

Similar to other tumors, the assessment of MSI in ovarian cancer has been highly variable. The reported incidence of MSI in invasive ovarian tumors ranges from ~5 to >50%, depending on the number and types of markers used as well as the definition of MSI (12–14, 30, 31). Our study is the first to apply the NCI criteria to assessment of MSI in ovarian cancers. We evaluated the utility of the five NCI recommended markers as well as nine additional markers. Our results indicate that inclusion of an additional marker, NME1, to the five NCI markers would have allowed detection of all of the MSI-H cases. On the basis of this panel, 13 (12%) tumors were found to have MSI-H.

The BAT26 locus contains a 26-repeat adenine tract and is located within the fifth intron of the MSH2 gene (22). The BAT25 locus contains a 25-repeat thymine tract located within intron 16 of the c-kit oncogene (32). The quasisomomorphic profiles of both BAT25 and BAT26 facilitate the identification of MSI, because shortened, unstable alleles can easily be differentiated from alleles of normal size. Some authors have suggested that given the small allelic profile at these two loci, MSI can be characterized without the need for matched, normal tissue DNA for comparison (22, 23). However, Pyatt et al. (24) reported that allelic variations can occur in ~13% at the BAT26 locus and ~18% at BAT25 in African-American individuals. In our population, only two tumors had polymorphic variation at the BAT25 locus. No variation was noted at the BAT26 locus. We routinely evaluated peripheral blood and tumor specimens on each patient for each marker to avoid mistakes in classifying an allelic variation as MSI.

Because of the variety of techniques used for assessment of MMR defects, there has been variability in the reported clinicopathological correlations of MSI in ovarian as well as other tumors (33–35). Survival benefits have been demonstrated for patients with HNPCC-related colon cancers with MSI (1, 36, 37). However, no differences in survival have been noted in tumors with MSI in sporadic colon (38, 39), endometrial (40), prostate (41), or gastric (42, 43) cancers. The differences in clinical outcome based on different tumor sites may reflect differences in somatic mutations in genes regulating the inhibitory and apoptotic pathways in these cancers (7, 44). In the present study, no significant differences in clinical outcome were detected based on MSI.

Six MMR genes have been discovered including MLH1, MSH2, PMS1, PMS2, MSH6 (GTBP), and MSH3 (19). It is possible that immunohistochemistry for these and potentially other yet unidentified genes may be useful alone or in conjunction with PCR-based MSI detection. The incidence of mutations in the DNA MMR genes appears to be lower in sporadic tumors with MSI-H (14, 45, 46). It is likely that other mechanisms, such as hypermethylation, may play a role in gene inactivation in these tumors (35, 47). Promoter methylation abnormalities in these genes may result in a MMR-deficient phenotype, which may affect clinical behavior and response to treatment (35). We are actively evaluating the role of promoter methyla-

| Table 2 Association of clinicopathological variables with MSI |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | MSS             | MSI-H           | MSI-L           | MSI-Any         | 100%           |
| Age              | 59.4 (± 12.3)   | 54.3 (± 9.9)    | 61.6 (± 11.5)   | 0.19 (± 11.4)   | 59.1 (± 8.4)   |
| Stage            |                |                |                |                |                |
| II/III           | 15 (3) 85 (5)   | 0.81 (8)        |                |                | 0.91           |
| III/IV           | 55 (10) 21 (31) |                |                |                |                |
| Grade            |                |                |                |                |                |
| I                | 11 (1) 0.54 (2) | 0.23 (3)        | 0.37           |                |                |
| II               | 24 (6) 6 (12)   |                |                |                |                |
| III              | 35 (6) 18 (24)  |                |                |                |                |
| Histology        |                |                |                |                |                |
| Serous           | 42 (7) 0.41 (21)| 0.06 (28)       | 0.22           |                |                |
| Other            | 28 (6) 5 (11)   |                |                |                |                |
| Ascites          |                |                |                |                |                |
| No               | 20 (2) 0.40 (5) | 0.35 (7)        | 0.22           |                |                |
| Yes              | 50 (11) 21 (32) |                |                |                |                |
| Cytoreduction    |                |                |                |                |                |
| Optimal          | 45 (8) 0.89 (16)| 0.80 (24)       | 0.78           |                |                |
| Suboptimal       | 25 (5) 10 (15)  |                |                |                |                |
| Median Survival (yr) | 4.44 (± 1.2)   | 4.56 (± 0.43)   | 3.17 (± 1.21)  | 0.39 (± 0.38)  |                |

Fig. 3. Results from the amplification of BAT25 from normal (N) and tumor (T) DNA. Both tumors demonstrate one allele within the range of size (upper bands) described previously and one polymorphic allele (lower band).
tion abnormalities in ovarian cancer. We conclude that MSI is relatively common in ovarian cancer. The role of MSI in ovarian cancer response to chemotherapy and clinical outcome needs to be defined further based on the techniques presented here as well as other techniques.

References


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