Frequency and Prognostic Impact of Microsatellite Instability in a Large Population-based Study of Endometrial Carcinomas

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ABSTRACT

The replication error repair (RER) phenotype has been reported in 9–43% of sporadic endometrial carcinomas, but there are conflicting data about its effect on prognosis in this disease. This study was performed to establish the frequency of the RER phenotype and to determine its effect on prognosis in a population-based series of 259 endometrial carcinomas with long-term follow-up. Five mononucleotide and dinucleotide microsatellite markers on different chromosomes were analyzed, and tumors exhibiting microsatellite instability at two or more loci were classified as RER+. A total of 116 of 259 tumors (45%) were RER+. The 5-year survival rate for the RER− group was 76.2% compared with 79.6% for RER+ cases (P = 0.6). The 5-year recurrence-free survival rate among the 228 patients surgically treated for cure was 80.6% in the RER− group compared with 83.6% in the RER+ group (P = 0.6). The analysis indicates that the RER phenotype is common in endometrial carcinomas, but there is no association with prognosis in this large population-based series of endometrial carcinomas. endometrial carcinomas collected from Hordaland County in Norway with the intention of definitively establishing the rate of MSI in endometrial carcinoma. To avoid selection bias and to reduce problems with external validity, we examined this 10-year population-based series with complete follow-up. Because we also aimed to determine the prognostic effect of MSI, we calculated that with an overall 5-year survival rate of 70% for endometrial cancer, a sample size of 300 tumors would give us 90% power to detect a 20% change in mortality in the RER+ group if 30% of tumors exhibited the RER phenotype.

MATERIALS AND METHODS

All patients diagnosed with endometrial carcinoma in Hordaland County, Norway during the 10-year period of 1981–1990 have been studied. Hordaland County has approximately 400,000 inhabitants, representing approximately 10% of the total Norwegian population, and has a similar age-adjusted incidence rate of endometrial cancer (19). Data on different patient characteristics, treatment, and follow-up collected for all 316 of the patients diagnosed during this period have been described previously (20, 21). All patients were retrospectively staged according to the FIGO 1988 criteria, and tumor specimens were graded and classified by a single pathologist (L. A. A.) according to WHO 1994 criteria. The treatment protocol for the period has been reported previously (20).

Follow-up. The median follow-up period for the survivors was 9 years (range, 4–15 years). None of the patients was lost due to insufficient follow-up data. Information about survival was obtained from the medical records and correspondence with the primary physician. The data were cross-checked with information from the Cancer Registry of Norway, which is matched against the Register of Deaths of Statistics Norway.

Tumor Specimens. A total of 316 women were diagnosed with primary endometrial carcinoma between 1981 and 1990 in Hordaland County. Twelve cases were excluded due to a change in diagnosis at reclassification. In five cases, the diagnosis was based on cytological examination only, with no histological specimens available. From the remaining 299 patients, paraffin blocks from the tumor specimen were available for further investigation in 286 of the cases (96%). Both tumor tissue and corresponding normal tissue were available from 259 of these patients (91%). Normal tissue and tumor tissue were microdissected using separate 5-µm formalin-fixed, paraffin-embedded slides from each case. Tissue was digested overnight at 56°C in a solution containing 10 µl of 1 M Tris, 0.4 µl of 0.5 M EDTA, 2 µl of 20 mg/ml proteinase K, and 87.6 µl of distilled water. Proteinase K was inactivated by boiling for 8 min. Extracted DNA was stored at 4°C.

MSI Analysis. Tumor and corresponding normal DNA from each case were analyzed using a panel of five markers for mononucleotide and dinucleotide repeat sequences (CA repeats, D10S187, D18S55, and D18S58; poly(A) repeats, BAT 26 and BAT 40). The markers on chromosome 18 and the mononucleotide markers were chosen because they had previously been analyzed in studies of MSI in colorectal or endometrial carcinomas. BAT 26 lies within the hMSH2 gene on chromosome 2. D10S187 was analyzed because it lies within the area of the PTEN gene that has been shown to be mutated in a high proportion of microsatellite unstable endometrial cancers.

PCR reactions were carried out in a 20-µl reaction containing 1.5 µmol of forward primer; 2 µmol of reverse primer; 0.5 µmol of 32P-labeled forward primer; 0.2 mm each of dATP, dGTP, dTTP, and dCTP; 0.25 unit of Taq Supreme enzyme (Helena Biosciences); and 1× PCR buffer. Thermal cycling was performed on an Omigene thermal cycler (Hybaid) using the following conditions: (a) 97°C (5 min); (b) five cycles of 95°C (45 s), annealing temperature (60 s), and 68°C (60 s); (c) 30 cycles of 95°C (45 s) and annealing temperature (45 s); and (d) a final extension step at 68°C (10 min). The PCR...
enzyme was added after the initial denaturation step. PCR products were separated on a 5% denaturing polyacrylamide gel (Sequagel, National Diagnostics/Flowgen) and visualized using autoradiography. MSI at any locus was determined by the presence of new alleles in the tumor as compared with the normal tissue. Tumors were defined as RER+ if instability was demonstrated in at least two of the five markers.

**Statistical Analysis.** The age at primary treatment, FIGO stage, histological type, and grade were compared between RER+ and RER− cases using the χ2 test. Differences were considered significant for P < 0.05. These traditional clinicopathological prognostic variables were also analyzed in relation to MSI at individual marker loci and the number of unstable loci overall. Univariate analyses of time to recurrence (recurrence-free survival) or time to death due to endometrial cancer (cause-specific death) were performed using the product-limit procedure (Kaplan-Meier method), with the time of primary operation as the entry date. Only those patients initially cured surgically were included for the estimation of recurrence-free survival; the occurrence of recurrent disease was defined as the event, whereas the patients not developing recurrent disease were censored at the date of the latest clinical examination. For the estimation of survival, death due to endometrial carcinoma was defined as the event, whereas the survivors were censored at the date of the latest clinical examination. Patients who died due to other causes were censored at the time of death. The Mantel-Cox test was used to compare the survival curves for different patient groups defined by categories of each variable. Data were analyzed using the SPSS software package.

Before commencing the analysis, it was calculated that with a reported overall 5-year survival for endometrial cancer of 70%, 300 tumors would need to be analyzed to achieve 90% power to detect a 20% change in survival due to the RER phenotype if 30% of the population were RER+.

**RESULTS**

**MSI Analysis.** Thirteen of 272 tumors (6%) were excluded from the analysis because RER status could not be definitively established. In these 13 tumors, MSI was demonstrated at one marker locus, but the results for one or more of the remaining loci in the panel could not be conclusively assessed. Of the remaining 259 endometrial carcinomas analyzed, 116 (45%) were RER+, exhibiting MSI at two or more loci. Results were available for all five markers in 225 of 259 cases (87%), of which 92 (41%) were RER+. The remaining 34 of 259 tumors had one missing marker result, but RER status could still be conclusively assessed, based on the available results. Table 1 shows the frequency of MSI at individual loci in all of the tumors analyzed, in those for which a full panel of results was obtained, and in tumors that were RER+. A total of 33 of 92 (36%) RER+ cases exhibited MSI at just two loci, 17 of 92 (18%) RER+ cases exhibited MSI at three loci, 18 of 92 (20%) RER+ cases exhibited MSI at four loci, and 24 of 92 (26%) RER+ cases were unstable at all five loci. Instability at the mononucleotide marker BAT 40 was the best predictor of the RER phenotype overall, with instability exhibited in 80% of all RER+ tumors. The traditional clinicopathological variables of prognostic importance in endometrial carcinoma are related to RER status in Table 2. There was no significant correlation between age, FIGO stage, histological type, or histological grade and overall RER status or MSI at individual marker loci. No correlation was found between the number of unstable loci and age at primary operation, histological grade, or FIGO stage. However, among the 225 tumors with all five markers available, none of the clear cell or serous papillary tumors were highly unstable (i.e., four or five positive markers) compared with 21% of the endometrioid/adenosquamous tumors (P = 0.03).

**Survival Analysis.** Fig. 1 shows the Kaplan-Meier curves for survival among the 259 cases analyzed and recurrence-free survival for the 228 of 259 patients surgically treated for cure. The 5-year survival rate was 76.2% for the RER− group compared with 79.6% for RER+ cases (P = 0.6). The 5-year recurrence-free survival rate among the 228 patients surgically treated for cure was 80.6% in the RER− group compared with 83.6% in the RER+ group (P = 0.6).

Neither of these outcomes was significantly influenced by MSI at any one marker locus when these were analyzed separately, nor were these outcomes significantly influenced by the proportion of markers demonstrating MSI.

**DISCUSSION**

The RER phenotype was demonstrated in 45% of the endometrial cancer cases in this population-based series. This RER phenotype frequency is somewhat higher than that reported in the majority of studies in the literature. There are several possible explanations for this finding. First, there may be RER+ endometrial cancers associated with germ-line DNA repair gene mutations and the HNPCC syndrome within the population. Details of family history were not collected specifically to exclude HNPCC cases, but HNPCC is a rare syndrome, and it is unlikely to have been a major factor in our population-based series. Second, we may have misinterpreted minor alterations in band pattern or equivocal shifts as MSI. To exclude this possibility, all results were classified independently by two investigators (N.D.M. and A.R.), and in cases where classification was difficult, DNA was reextracted, and the PCR was repeated to confirm that the observed result was not a PCR artifact. Third, the combination of markers used in our study may demonstrate RER status more reliably than combinations used in other studies. The panels of markers used in the majority of studies of MSI in endometrial cancer have been limited to dinucleotide markers (7, 8, 9, 11, 13). Mononucleotide repeats are known to be more susceptible to replication errors than dinucleotide repeats (2, 17), and longer mononucleotide sequences are more often unstable than shorter sequences. Our panel of markers is therefore more likely to give an accurate assessment of the frequency of RER than previous studies of endometrial cancer.

Because this is the first reported population-based series of endo-

### Table 1. Frequency of MSI at individual loci among all tumors analyzed, tumors with results for all five microsatellite markers, and in RER+ tumors with results for all five markers

<table>
<thead>
<tr>
<th>Microsatellite marker</th>
<th>All tumors analyzed</th>
<th>Tumors with results for all microsatellite markers (n = 225)</th>
<th>RER+ tumors with five marker results (n = 92)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% MSI at each locus</td>
<td>% MSI at each locus</td>
<td>% MSI at each locus</td>
</tr>
<tr>
<td>D10S187</td>
<td>75/259 (29%)</td>
<td>60 (27%)</td>
<td>53 (58%)</td>
</tr>
<tr>
<td>D18S55</td>
<td>84/256 (33%)</td>
<td>68 (30%)</td>
<td>62 (67%)</td>
</tr>
<tr>
<td>D18S58</td>
<td>97/251 (42%)</td>
<td>95 (42%)</td>
<td>66 (72%)</td>
</tr>
<tr>
<td>BAT 26</td>
<td>74/257 (29%)</td>
<td>62 (28%)</td>
<td>56 (61%)</td>
</tr>
<tr>
<td>BAT 40</td>
<td>107/256 (42%)</td>
<td>86 (38%)</td>
<td>74 (80%)</td>
</tr>
</tbody>
</table>

### Table 2. Patient characteristics according to RER status in 259 patients with endometrial carcinoma

<table>
<thead>
<tr>
<th>Variable</th>
<th>RER− % (n = 143)</th>
<th>RER+ % (n = 116)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at primary operation</td>
<td>64 (44.8%)</td>
<td>65 (56.0%)</td>
<td>0.071</td>
</tr>
<tr>
<td>&lt;64.7 years</td>
<td>79 (55.2%)</td>
<td>51 (44.0%)</td>
<td>0.1</td>
</tr>
<tr>
<td>FIGO stage</td>
<td>106 (54.4%)</td>
<td>89 (45.6%)</td>
<td>1.0</td>
</tr>
<tr>
<td>I</td>
<td>6 (35.3%)</td>
<td>11 (64.7%)</td>
<td>0.009</td>
</tr>
<tr>
<td>II</td>
<td>21 (60.0%)</td>
<td>14 (40.0%)</td>
<td>0.071</td>
</tr>
<tr>
<td>III</td>
<td>9 (81.8%)</td>
<td>2 (18.2%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Histological type</td>
<td>129 (55.1%)</td>
<td>105 (44.9%)</td>
<td>0.9</td>
</tr>
<tr>
<td>Endometrioid/adenosquamous/adenocanthoma</td>
<td>14 (56.0%)</td>
<td>11 (44.0%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Clear cell/serous papillary</td>
<td>30 (50.8%)</td>
<td>29 (49.2%)</td>
<td>0.6</td>
</tr>
<tr>
<td>Histological grade</td>
<td>86 (56.6%)</td>
<td>66 (43.4%)</td>
<td>0.6</td>
</tr>
<tr>
<td>Well differentiated (grade 1)</td>
<td>27 (56.3%)</td>
<td>21 (43.8%)</td>
<td>0.6</td>
</tr>
<tr>
<td>Moderately differentiated (grade 2)</td>
<td>129 (55.1%)</td>
<td>105 (44.9%)</td>
<td>0.9</td>
</tr>
<tr>
<td>Poorly differentiated (grade 3)</td>
<td>64 (44.8%)</td>
<td>65 (56.0%)</td>
<td>0.071</td>
</tr>
</tbody>
</table>

* a χ2 test.  
* b Median age at primary operation was used as cut point.  
* c Information about FIGO stage is missing for one case.
other study has reported a better prognosis for RERb as transforming growth factor 1 mutations that commonly accumulate in RER. A good prognosis (1). This may be explained by the fact that somatic RER also contrasts with evidence that RER prognostic influence of RER status in our study of endometrial carcinoma selection, follow-up, and statistical methods applied. The absence of studies are difficult to compare, however, due to differences in patientRERS.

metrial carcinomas to be analyzed using widely accepted criteria for determining RER status, the higher frequency of the RER phenotype demonstrated is likely to represent the true prevalence of the RER phenotype in endometrial carcinomas.

The lack of prognostic influence of RER status in our study conflicts with the findings of one smaller previous study (7) in which patients with RER+ tumors had a higher mortality than those with RER− tumors. One other study has reported a better prognosis for RER+ endometrial cancers, but the survival difference was not statistically significant (9).

The studies are difficult to compare, however, due to differences in patient selection, follow-up, and statistical methods applied. The absence of prognostic influence of RER status in our study of endometrial carcinomas also contrasts with evidence that RER+ colorectal carcinomas have a good prognosis (1). This may be explained by the fact that somatic mutations that commonly accumulate in RER+ colorectal cancers, such as transforming growth factor βRII, insulin-like growth factor II, and BAX mutations, do not occur at such high frequencies in RER+ endometrial cancers (5, 16); therefore, the inhibitory and apoptotic pathways may behave differently in these cancers. Alternatively, these genes may not play such a large role in the tumorogenesis of endometrial cancers as they do in colorectal cancers, so that mutations within them have less effect on tumor behavior and patient outcome. It is of interest that parallel studies of hMLH1 and Pten methylation performed in our population of endometrial cancer cases suggest that although these events are positively associated with the RER phenotype, neither event is significantly associated with prognosis. The RER phenotype, hMLH1 methylation, and abnormal hMLH1 expression were more common in diploid tumors and in those with normal p53 expression, whereas Pten methylation corre-

lated with metastatic disease.4,5 These findings are consistent with the data presented here, which suggest that although MSI does not determine prognosis in endometrial cancer, distinct pathways of carcinogenesis exist between RER+ and RER− endometrial cancers.

In conclusion, the RER phenotype is common in endometrial carcinomas but is not significantly associated with either traditional clinicopathological variables or prognosis in our large population-based series with long and complete follow-up.

ACKNOWLEDGMENTS

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B
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