Replication Errors in Benign and Malignant Tumors from Hereditary Nonpolyposis Colorectal Cancer Patients


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Abstract

A replication error (RER) phenotype has been documented both in sporadic colorectal tumors and in tumors from patients with hereditary nonpolyposis colorectal cancer (HNPPC). In the current study the data of 49 (16%) sporadic colorectal cancers (CRCs) and 29 (86%) CRCs from HNPPC patients were found to be RER+. All 9 (100%) CRCs from HNPPC patients with germline mutations of the mismatch repair gene MSH2 were found to be RER+, while 16 of 20 CRCs from HNPPC kindreds unrelated or not studied for linkage to MSH2 were RER+. Corresponding analysis in colorectal adenomas revealed that only 1 of 33 (3%) sporadic tumors but 8 of 14 (57%) HNPCC tumors were RER+. Moreover, RER was found in all 6 extracolonic cancers (endometrium, 2; kidney, 1; stomach, 1; duodenum, 1; and ovary, 1) derived from members of HNPCC families. These data suggest the involvement of mismatch repair deficiency in the premalignant stage of tumorigenesis in HNPPC cases, and suggest that mismatch repair genes (MSH2 or others) are defective in the germline of nearly all these patients.

Introduction

Widespread alterations in simple repeated sequences have been found in several tumor types (1–6). Such alterations are most easily observed as changes in the lengths of microsatellite sequences in tumor DNA as compared with normal DNA from the same individual. The alterations have been shown to involve mono-, di-, tri-, and tetranucleotide repeats (1–3, 7), and tumors with these abnormalities have been called RER+. Recently the RER phenotype was found to be associated with deficient strand-specific correction of slipped-strand and base-base mismatches, establishing it as a product of a mismatch repair defect (8).

The RER phenomenon is characteristic of colorectal cancers developing in families with HNPPC (3) but was initially noted in “sporadic” patients (1, 2, 9). This raises the possibility that it could be a result of either germline or somatic mutations. A gene on chromosome 2p (human MSH2) involved in mismatch repair was recently cloned (10, 11) and found to be mutated in the germline of affected members of HNPPC families (11). Approximately one-half of HNPPC is caused by mutations of MSH2, as inferred from linkage and sequencing analyses (3, 11, 12). The remaining cases of HNPPC are caused by other genes, at least one of which appears to be on chromosome 3p (13).

Colorectal cancer arises as a multistep process involving mutations in oncogenes and tumor suppressor genes as normal epithelium evolves through different adenomatous stages to cancer and finally metastasis (14). The role of mismatch repair defects in this process must be clarified. In this paper we address five issues: (a) we hypothesize that if a RER is due to deficient mismatch repair in vivo, it should occur in all CRCs of patients with germline mutations of MSH2; (b) if the other tumor types characteristic of patients with MSH2 mutations are caused by defective mismatch repair, then these tumors should manifest the RER phenotype; (c) if HNPPC in general is caused by defective mismatch repair genes, then CRCs occurring in HNPPC kindreds unrelated to MSH2 should also be RER+; (d) if mismatch repair defects are involved in the relatively early stages of colorectal tumorigenesis, then the RER phenotype should be found in benign as well as malignant tumors; (e) we wished to establish whether previously observed right sided predominance of RER+ sporadic CRCs was more marked than for HNPPC tumors. If this were the case it might indicate site-specific mechanisms of pathogenesis.

Materials and Methods

Sporadic tumors (49 CRCs and 33 adenomas) were those in which no family history suggestive of HNPPC was noted at the time of diagnosis. We could not exclude that a subset of these cases was actually from HNPPC kindreds, but this is typical of those cancers encountered in routine clinical practice. The HNPPC families studied here met the stringent criteria for HNPPC set by the International Collaborative Group on HNPPC (15): (a) at least three relatives should have histologically verified colorectal cancer, and one of them should be a first degree relative to the other two; (b) at least two successive generations should be affected; and (c) in one of the relatives colorectal cancer should be diagnosed before the subject is 50 years of age. Eighteen tumors were from families J or C (12) in which germline mutations of MSH2 have been described (11). The other HNPPC tumors occurred in patients from kindreds in which linkage analysis excluded MSH2 as a causative factor (n = 9) or from kindreds where linkage analysis was uninformative or impossible to perform (n = 22) (3).

Ten CRCs and one colorectal adenoma have been analyzed previously for RER (3). To determine RER status of the remaining cases DNA from normal and tumor tissue was genotyped with seven microsatellite markers: D2S136, DSS404, D7S519, D8S255, D15S120, D17S787, and D20S100 (16). Additional markers (D1S216, D11S904, D13S179 and D19S179) (16) were occasionally used. When frozen tumors were available, DNA was purified from cryostat sections of tissue microdissected to remove most nonneoplastic cells, as described previously (17). DNA from normal colonic mucosa or blood samples from unaffected relatives was used as a control.
leukocytes was purified by standard methods. To study DNA extracted from paraffin-embedded formalin-fixed samples ["protocol A" described by Kallio et al. (18)], primers that produce fragments approximately 100 bases long were also used. These markers were D4S422, D6S294, D7S520, D9S178, D12S83, D13S158, D14S73, D18S57, D20S95, and D22S284 (16). The methodology for analyzing such microsatellite markers has been described previously (12). One sample pair consisted of a carcinoma and an adenoma because no normal tissue was available (Fig. 1, patient 1). Results were obtained with 5–14 markers in all samples. If two or more markers showed alterations in the allele sizes of the tumor DNA the tumor was categorized as RER+. Thus, the RER− category consisted of tumors with alterations detected in 0 or 1 locus.

**Results**

Among sporadic CRCs 8 of 49 (16%) were RERs (Table 1). In these 8 tumors 60% (SD = 23%) of the microsatellite markers tested exhibited alterations. Of the 29 CRCs derived from HNPCC patients, 25 were RERs. In these 25 tumors, 57% (SD = 26%) of the markers exhibited alterations. Of the 29 CRCs emanating from HNPCC kindreds, 9 belonged to pedigrees with germline mutations of MSH2 [families J and C (11, 12)]. All 9 of these cancers (100%) were RER+ (Table 1). In the other 20 cancers, linkage analysis either gave evidence against germline involvement of MSH2 (n = 7) or was uninformative or not performed (n = 13) (3). Sixteen of these 20 CRCs were RER+, including 6 tumors where involvement of MSH2 was excluded. We next studied 33 sporadic adenomas from 25 patients. Only one (3%) was RER+ (Fig. 1, Table 1). In contrast, of 14 adenomas emanating from HNPCC family members, 8 (57%) were RER+ (Fig. 1; Table 1). Four were from families C or J (with germline mutations of MSH2) and three of these four were RER+ (Table 1).

There were at least two differences between HNPCC patients and the sporadic patients with RER+ tumors: (a) the age of the 8 sporadic patients (mean age at diagnosis, 69 years; youngest patient, 56 years) was significantly higher than that of the average HNPCC patients [mean age at first CRC diagnosis, 40–45 years (19–22)]. This is consistent with previously published data (1–3, 23); (b) five of the eight sporadic CRCs exhibiting RER occurred in the proximal portion of the large bowel (prior to the splenic flexure). In previously reported series, 56 of 68 sporadic CRCs exhibiting RER were proximal (Table 2).

### Table 1 Frequency of microsatellite instability (RER) in colorectal tumors

<table>
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<th></th>
<th>Total</th>
<th>RER−</th>
<th>RER+</th>
</tr>
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<tr>
<td>Sporadic carcinomas</td>
<td>49</td>
<td>41</td>
<td>8 (16%)</td>
</tr>
<tr>
<td>All HNPCC carcinomas</td>
<td>29</td>
<td>4</td>
<td>25 (86%)</td>
</tr>
<tr>
<td>Carcinomas from MSH2-linked families</td>
<td>9</td>
<td>0</td>
<td>9 (100%)</td>
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<tr>
<td><strong>Sporadic adenomas</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All HNPCC adenomas</td>
<td>33</td>
<td>32</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Adenomas from MSH2-linked families</td>
<td>14</td>
<td>6</td>
<td>8 (57%)</td>
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<table>
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<tr>
<th></th>
<th>Total</th>
<th>RER−</th>
<th>RER+</th>
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<tr>
<td><strong>Sporadic adenomas</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All HNPCC adenomas</td>
<td>14</td>
<td>6</td>
<td>8 (57%)</td>
</tr>
<tr>
<td>Adenomas from MSH2-linked families</td>
<td>4</td>
<td>1</td>
<td>3 (75%)</td>
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* Numbers in parentheses, percentage.

1 Numbers in parentheses, percentage.

**Discussion**

This study confirmed the hypothesis that a mutation in the MSH2 gene causes a RER phenotype because all carcinomas from patients with germline mutations of this gene were RER+. Altogether, 25 of 29 of the studied CRCs from HNPCC kindreds were RER+, establishing a strong association between HNPCC and RER regardless of the underlying germline mutation.
We show here for the first time that RER is common in adenomas derived from patients with HNPCC. The fact that all adenomas were not RER+ is understandable, as all adenomas found in members of HNPCC kindreds are not likely to be related to the susceptibility gene. In fact, in six kindreds linked to the MSH2 locus, only five of nine individuals with adenoma as the only sign of HNPCC had a haplotype associated with the cancer phenotype (data not shown). Our results support the hypothesis that the RER phenotype reflects the basic defect in HNPCC tumors. The findings provide strong evidence that adenomatos polyps are precursors for CRCs in HNPCC patients, setting a rationale for the efficiency and desirability of screening of susceptible individuals by colonoscopy. Furthermore, studying adenomas for RER+ could prove to be useful in the diagnosis of HNPCC, especially in kindreds without known germline mutations. However, it is important to note that at least one adenoma from an individual with a germline mutation of MSH2 (as inferred from extensive haplotype analysis) was RER−. Another adenoma from this same individual was RER+. This suggests that the RER phenotype is not necessary for adenoma formation in HNPCC patients. It is more likely that the genetic instability created by RER+ accelerates the adenoma-carcinoma transition once adenomas are formed.

Our results on the RER status of sporadic adenomas confirm the finding of Young et al. (26) that RER+ is rare in such tumors. We found only 1 RER+ adenoma of 33 tested. Interestingly the patient with this adenoma had three other colorectal tumors (two adenomas and one carcinoma), all of which were RER−. These data could be interpreted to suggest that a mismatch repair gene was somatically mutated only in the RER+ adenoma from this patient, and that she had no germline mutation. In contrast, another patient with four colorectal tumors has been described in which all (two adenomas and two carcinomas) were RER+, suggesting a germline component (1). It will be of great interest in the future to determine what fraction of patients with sporadic RER+ tumors have germline, as opposed to strictly somatic, mutations of mismatch repair genes.

It was important that all six extracolonic cancers (from endometrium, stomach, ovary, kidney, and duodenum) from HNPCC patients were RER+. This suggests that the entire spectrum of tumorigenesis in HNPCC tumors (Lynch syndrome tumor spectrum) is related to an underlying defect in mismatch repair. Our results are consistent with those of Risinger et al. (5) who found that endometrial cancers from HNPCC kindreds were usually RER+. These investigators also found that at least one kindred exhibited significant linkage to markers on chromosome 3p. In our study, 16 of 20 carcinomas and 5 of 10 adenomas from non-MSH2-linked or uninformative kindreds were RER+. Taken together, the results strongly argue that mismatch repair genes are generally responsible for HNPCC, and that mismatch repair genes other than MSH2 will be found to be altered in the germline of HNPCC kindreds unlinked to chromosome 2.

Of previously reported sporadic RER+ CRCs (1–3, 23, 26), only 12 of 68 were distal. Relatively low age at onset, family history positive for cancer, or metachronous tumors were reported in 5 of these 12 cases (1, 23, 26). Of the eight sporadic RER+ CRCs studied here, three were distal. One of these patients had a mother who had died at age 73 years of uterine cancer, a tumor characteristic of HNPCC (20–22). Thus, altogether at least 6 of the 15 distal sporadic RER+ cases studied thus far are suggestive of a hereditary component. One of the proximal RER+ -sporadic cases detected in this study turned out to have a family history of intraabdominal tumors. Altogether, of the 61 proximal CRCs exhibiting RER from sporadic patients, only 5 had any reported evidence of a familial basis (1–3, 23, 26, and this study).

The combined results suggest that tumors in the distal part of the colorectum which exhibit RER are often indicative of a germline mutation in a mismatch repair gene. To explain the more preponderant right-sidedness of RER+ tumors in sporadic than in HNPCC patients we hypothesize that in addition to inherent site-specific differences between cells, environmental factors such as the occurrence of carcinogens might be involved. More studies are obviously needed to better define the role of environmental and genetic factors in RER+ colorectal tumors, regardless of their location in the bowel, and in RER+ tumors in other organs.

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References

4. Perou, C., and McCall, J. C. The human MSH2 gene is a "housekeeping" gene that is expressed in all tissues thus far analyzed (10, 11). Why tumors from patients with germline mutations of this gene should be limited to a few tissue types is enigmatic and provocative.
5. Acknowledgments
6. We thank Lea Pyllkänen, Kirsi Hopponen, Tuula Lehtinen, Sinikka Lindh, Sharon M. Stewart, Susan Booker, Jennifer Cavaleri, Jane Lynch, Sue Slo-minski-Caster, Tom Smyrk, and Steve Landspa for assistance with samples and information.
7. References
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