Microsatellite Instability Is a Predictive Factor of the Tumor Response to Irinotecan in Patients with Advanced Colorectal Cancer

David Fallik, Francesco Borrini, Valérie Boige, Jérôme Viguier, Sandrine Jacob, Catherine Miquel, Jean-Christophe Sabourin, Michel Ducrœux, and Françoise Praz

ABSTRACT

The aim of our study was to assess the relationship between colorectal tumor responsiveness to irinotecan and microsatellite instability (MSI), a feature of colorectal tumors with DNA mismatch repair defect. Seventy-two patients with metastatic colorectal cancer were included in our retrospective study. A complete response to irinotecan was observed in 1 patient and a partial response in 10 patients, whereas 61 patients did not respond to this treatment. We analyzed the protein expression of hMLH1, hMSH2, and BAX by immunohistochemistry, determined the MSI phenotype, and looked for mutations in the coding repeats located in the transforming growth factor β-RII, BAX, hMSH3, and hMSH6 genes. All 44 tumors analyzed expressed detectable levels of hMLH1; 1 tumor lacked hMSH2 staining, whereas 4 tumors showed a marked decrease in BAX expression. A better response to irinotecan was observed in the patients whose tumors have lost BAX expression (P < 0.001). Among the 7 tumors that displayed a MSI-H phenotype, 4 responded to irinotecan, whereas only 7 of the 65 MSI-L/microsatellite stable tumors did (P = 0.009). Seven of the 72 tumors had inactivating mutations in the coding repeats of the target genes. Three tumors displayed a mutation in the poly-A10 tract of the transforming growth factor β-RII gene, associated with a 1-bp deletion in the poly-AT repeat of hMLH1 in one tumor and with a 1-bp deletion in the poly-G8 tract of BAX in another. Four tumors displayed mutations in the poly-G8 repeat of BAX, 2 mutations in hMSH6 and hMSH3 were characterized. Among the 7 tumors with mutations in these target genes, 5 responded to irinotecan, whereas only 6 of the other 65 tumors did (P < 0.001), indicating that MSI-driven inactivation of target genes modifies tumor chemosensitivity. Our observations allowed us to define the first useful predictive criteria for irinotecan response in patients with colorectal cancer.

INTRODUCTION

CRC is one of the most common adult malignant tumors affecting 1 person of 20 in Northern America and Western Europe. Although about half of the patients may be cured with surgery, many will develop metastatic disease, which necessitates chemotherapeutic treatments (1). Fluorouracil modulated with folinic acid has been the most extensively used first-line treatment for metastatic CRC, but objective response to 5-FU is observed in only 20–30% of the patients (2). The antitumor activity of IRI, an analogue of CPT, has been documented in 15–30% of patients with metastatic CRC after 5-FU failure (3, 4).

Like CPT, IRI interferes with the catalytic cycle of the nuclear enzyme topoisomerase I by stabilizing the covalent complex formed between the DNA and enzyme (5). This results in an increase in the number of single-strand breaks, as well as an inhibition of both replication and transcription. The single-strand breaks may be converted into DSBs after replication fork collision (5). In a recent study, we have investigated the possible involvement of the DNA MMR system in the cytotoxicity of topoisomerase inhibitors and have shown that CRC cell lines defective in MMR exhibit increased sensitivity to CPT (6).

The MMR system is best known for its role in postreplicative repair where it recognizes and repairs mismicrosorpaired bases, as well as small insertion or deletion loops arising during DNA replication (reviewed in Refs. 7–9). In addition to mutation avoidance, some of the MMR components participate in various DNA repair processes, including DSB repair and recombination (reviewed in Ref. 10). Furthermore, some MMR proteins have been involved in cell cycle regulation and the induction of apoptosis in response to a variety of DNA lesions (11–13). In human cells, mismatch recognition is performed by hMSH2 heterodimerized either with hMSH6 for base-base mismatches and loops of one or a few nucleotides or with hMSH3 for insertion/deletion of two or more extrahelical bases. Once bound to mismatches, these complexes interact with another heterodimeric complex, composed of hMLH1 and hPMS2 (14).

Germ-line alterations of MMR genes, usually hMSH2 or hMLH1, cause susceptibility to HNPCC, a genetic disorder that accounts for ~5% of all cases of CRC (Refs. 15–17 and reviewed in Refs. 9, 18–20). In HNPCC tumors, inactivation of the wild-type allele of the inactive MMR gene most often results from loss of heterozygosity or somatic mutation (21, 22). These tumors which display biallelic inactivation of one of the MMR genes are characterized by high levels of MSI, defined by the accumulation of mutations, mostly insertions or deletions in short tandem repeats throughout the genome. In addition, the MSI phenotype is not confined to HNPPC tumors but also occurs in ≤15% of sporadic CRC (23–25). Most of the mutations arising in MSI tumor cells are located in untranslated intergenic or intronic sequences. Yet, a number of genes whose sequence contains short cMNRs have been reported to be frequently affected in colorectal MSI tumors. Among the possible targets are genes that encode proteins involved in signal transduction (TGFβ-RII), apoptosis (BAX), DNA repair (hMSH3 and hMSH6), transcription regulation, or inflammatory response (reviewed in Refs. 20 and 26).

Given the incidence of the MSI phenotype among CRCs, the fact that a defect in MMR results in hypersensitivity to CPT may be particularly relevant to the treatment of CRC (6). Specific predictive criteria for IRI activity are crucially lacking. The aim of our study was to further investigate the relationship between the MSI phenotype, the inactivation of target genes, the loss of MMR protein expression, and the response to IRI in patients with metastatic CRC.
Materials and Methods

Patients and Tumors. Seventy-two patients with advanced metastatic CRC, whose disease had progressed under first-line 5-FU-based therapy, were included in the present study. All patients were given IRI (Campto®, Aventis, Antony, France) until disease progression or unacceptable toxicity effects came out. The chemotherapy regimen consisted of 300–350 mg/m² IRI infused alone once every 3 weeks or 180 mg/m² IRI combined with fluorouracil once every 2 weeks. The end point was the tumor response to chemotherapy with IRI defined according to the WHO criteria (27). The “responder” group included the patients with either a complete response (disappearance of the disease) or a partial response to the treatment (reduction of tumor volume of ≥50%). The “nonresponder” group included patients with stabilized tumors (volumetric reduction < 50% or enlargement ≥25%), as well as progressive tumors (size enlargement > 25% or appearance of new lesions). The tumor was located either proximal (ascending and transverse colon) or distal (descending and sigmoid colon) to the splenic flexure or in the rectum. The clinicopathological characteristics of the patients included in our study are shown in Table 1. The grading of differentiation was performed on the primary tumors according to the WHO criteria. Tumors were classified as mucinous when an area of extracellular mucin secretion was >50%. The tumor samples analyzed have been obtained from patients after failure of the first-line 5-FU-based therapy, before the second-line therapy.

Immunohistochemical Analysis. BAX immunostaining was performed on the first 44 tumors included in our series. hMLH1 and hMSH2 was analyzed on the same 44 tumors and the two additional MSI tumors (#115 and #150). IHC for hMSH6 and hPMS2 was restricted to the MSI tumors. Four-micrometer sections from tissues fixed either in formalin or in Bouin and embedded in paraffin were mounted onto glass slides, deparaffinized in xylene, and rehydrated through a graded alcohol series to distilled water. Antigen retrieval was performed by immersing the slides in a 10 mM citrate buffer (pH 6.0 for hMLH1 and BAX and pH 7.0 for hMSH2, hMSH6, and hPMS2), and heating them in a microwave oven for 30 min at 95°C. Sections were then incubated with mouse monoclonal antibodies against BAX (A3533, 1:150 dilution; Santa Cruz Biotechnology, Santa Cruz, CA), hMSH2 (clone NA27, 1:30 dilution; Oncogene Research, Cambridge, MA), hMSH6 (clone 44, 1:200; BD PharMingen, San Diego, CA), and hPMS2 monoclonal antibodies against hMLH1 (clone G168-15, 1:50 dilution; BD PharMingen). After a 5-min incubation at 95°C, amplification was achieved by 35 cycles consisting of 1-min denaturation at 95°C, 1-min annealing at 55°C, and 1-min elongation at 72°C, followed by a final 5-min elongation step at 72°C. A second round of PCR comprising 5–10 cycles was performed on 1/20 PCR1 product using sense primer labeled with 6-FAM, NED, or HEX fluorescent dye. After a 5-min heating at 95°C, mixtures of PCR products (1 μl), formamide-loading buffer, and ROX-labeled molecular weight markers (GS-400HD-ROX; Applied Biosystems) were loaded onto a denaturing 4.75% polyacrylamide/8 M urea gels and run at 1600 V for 6 h, using an ABI373 automated fluorescent DNA sequencer. Fragment sizes were determined using the GeneScan Analysis software. PCR detecting abnormal products was repeated twice to confirm the results.

Analysis of hMSH2 Exons. PCR was performed on genomic DNA using primers located at the intron-exon boundaries of the 16 hMSH2 exons, as described (29).

MSI Phenotype Analysis. The two noncoding quasi-monomorphic BAT25 and BAT26 mononucleotide microsatellites were analyzed using the primers published previously labeled with 6-FAM and NED, respectively (30, 31). The three dinucleotide markers D2S123, D5S346, and D17S250 were analyzed as described using primers labeled with 6-FAM, HEX, and HEX, respectively (32). Tumors were classified as MSI-L, if two or more of the five markers did. A single round of PCR was achieved by a 15-min denaturation step at 95°C, which allowed Taq polymerase activation, followed by 50 cycles of 30 s at 96°C, 30 s at 50°C, and 30 s at 72°C using 1 unit of HotStarTaq DNA Polymerase (Qiagen). Fluorescent PCR products were mixed with formamide and GS-HD400-RX molecular weight standards and run on a short capillary containing GS Performance Optimized Polymer 4, at a voltage of 15 kV on the ABI 310 Genetic Analyzer.

Statistical Analysis. The associations between the tumor response to IRI, the defect in BAX expression, and the cMNR-MSI or MSI phenotypes were assessed using Fisher’s exact test.

Results

Patient Selection and Tissue Samples. Seventy-two patients with CRC were included in the present series, 41 males and 31 females with a mean age of 53.6 ± 10.9 year (range 26–80). Patient clinical characteristics are given in Table 1. All these patients received IRI as second- or third-line treatment after disease progression on 5-FU-based chemotherapy. In our series, the responder group was constituted by the only patient (1.4%) who showed a complete response to IRI and the 10 patients (13.9%) with a partial response. The nonresponder group included the 29 patients (40.3%) with stabilization and the 32 patients (44.4%) with progressive disease. Twenty-two (30.6%) tumors were located in the proximal colon, 36 (50%) in the distal colon, and the remaining 14 (19.4%) in the rectum. Tumor specimens were obtained from 44 surgically resected large bowel primary tumors, 9 local peritoneal metastasis, and 19 hepatic metastases. Of the 44 primary CRC, 19 were well differentiated (G1), 17 were moderately differentiated (G2), and 3 were poorly differentiated (G3), 5

Table 1  Clinical and histological characteristics of the patients with advanced colorectal cancer treated with irinotecan

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>n</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis (yr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤50 yr</td>
<td>45</td>
<td>62.5</td>
</tr>
<tr>
<td>&lt;50 yr</td>
<td>27</td>
<td>37.5</td>
</tr>
<tr>
<td>Mean ± SE (range)</td>
<td>53.6 ± 10.9 (26–80)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>41</td>
<td>56.9</td>
</tr>
<tr>
<td>Female</td>
<td>31</td>
<td>43.1</td>
</tr>
<tr>
<td>Primary tumor site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>14</td>
<td>19.4</td>
</tr>
<tr>
<td>Distal colon</td>
<td>36</td>
<td>50</td>
</tr>
<tr>
<td>Proximal colon</td>
<td>22</td>
<td>30.6</td>
</tr>
<tr>
<td>Degree of differentiation*</td>
<td>19</td>
<td>43.2</td>
</tr>
<tr>
<td>Well</td>
<td>17</td>
<td>38.6</td>
</tr>
<tr>
<td>Moderate</td>
<td>3</td>
<td>6.8</td>
</tr>
<tr>
<td>Poor</td>
<td>5</td>
<td>11.4</td>
</tr>
</tbody>
</table>

* The degree of differentiation is only indicated for the 44 primary tumors.
Expression of hMLH1, hMSH2, hMSH6, and hPMS2 and Response to Chemotherapy with IRI. Protein expression for hMLH1 and hMSH2 was examined in 46 tumors. Both hMLH1 and hMSH2 were normally expressed in the nuclei of normal colonic mucosa and confined in crypt epithelial cells, stromal cells, and lymphocytes. One tumor exhibited an absence of nuclear staining of hMSH2 (#34) with positively stained stromal cells in the surroundings (data not shown). This tumor was poorly differentiated and arose in the proximal colon in a young patient who had a familial history of CRC. This hMSH2-negative tumor responded to the treatment with IRI. The expression of hMLH1 was detectable in all 46 tumors analyzed, but two tumors displayed low hMLH1 immunostaining intensity; one corresponded to a primary lesion, and the other was a hepatic metastasis. Both tumors primarily arose in the distal colon and were moderately differentiated. These 2 hMLH1-low tumors were observed in patients over 50 years and did not respond to IRI. The immunohistochemical detection of hMSH6 and hPMS2 has been performed for tumors that displayed MSI. The quality of staining with these antibodies was suboptimal for two archival samples (#31 and #34), most probably because of poor preservation. Among the six tumors that could be assessed, none displayed an unambiguous complete loss of expression of either hMSH6 or hPMS2 (data not shown).

Detection of hMSH2 Exonic Deletion. To determine whether hMSH2 loss of expression in tumor #34 resulted from an hMSH2 genomic deletion, we have performed PCR on genomic DNA using primers located at the intron–exon boundaries, as described (29). No genomic deletion could be identified through the screening of all 16 exons of hMSH2 (data not shown).

Expression of BAX and Response to Chemotherapy with IRI. BAX was normally expressed in the apical portion of cytoplasm of normal colonic epithelial cells and cytoplasm of lymphocytes. BAX-positive immunostaining of tumor cells was detected in 40 of the 44 cases analyzed by IHC (Fig. 1A). Yet, both the ratio of positively stained cells and intensity of BAX staining were heterogeneous on the same slide. Four tumors definitely lacked detectable expression of BAX, with the adjacent nontumoral tissue being positively stained. The staining pattern of one of the BAX-negative tumors (#34) is shown in Fig. 1B. Two of them corresponded to hepatic metastases and one was a peritoneal metastasis, whereas the fourth specimen was a primary rectal lesion. Interestingly, a partial response to IRI was achieved in all 4 BAX-negative tumors, whereas among the 40 tumors that expressed normal levels of BAX, only 3 patients experienced tumor regression (Table 2). Statistical analysis indicated that the loss of BAX expression is a predictive marker for the response to IRI chemotherapy in metastatic CRC (P < 0.001).

Association of MSI with Response to Chemotherapy with IRI. Tumor MSI analysis was first performed using two quasi-monomorphic mononucleotide repeats, BAT25 and BAT26 (Table 3). The tumors that displayed instability in either mononucleotide markers were further analyzed using the three dinucleotide repeats of the Bethesda panel and classified according to the National Cancer Institute recommendations (31). Among the 72 tumors, 7 (9.7%) displayed

Table 2 Relationship among BAX protein expression, frameshift mutation in the BAX poly-G8 tract, and the response of metastatic colorectal cancer to the treatment with irinotecan

<table>
<thead>
<tr>
<th>Case</th>
<th>Primary tumor site</th>
<th>Tumor specimen</th>
<th>Irinotecan tumor response</th>
<th>Bax Immunostaining</th>
<th>BAX-G8 mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Rectum</td>
<td>Primary tumor</td>
<td>Partial regression</td>
<td>Not detectable</td>
<td>Wild-type(^b)</td>
</tr>
<tr>
<td>31</td>
<td>Distal</td>
<td>Metastasis</td>
<td>Partial regression</td>
<td>Not detectable</td>
<td>Mutant</td>
</tr>
<tr>
<td>34</td>
<td>Proximal</td>
<td>Metastasis</td>
<td>Partial regression</td>
<td>Not detectable</td>
<td>Mutant</td>
</tr>
<tr>
<td>37</td>
<td>Distal</td>
<td>Metastasis</td>
<td>Partial regression</td>
<td>Not detectable</td>
<td>Mutant</td>
</tr>
<tr>
<td>150</td>
<td>Proximal</td>
<td>Primary tumor</td>
<td>Stable disease</td>
<td>nd</td>
<td>Mutant</td>
</tr>
</tbody>
</table>

\(^a\) The primary tumor site is proximal for tumor originating in the ascending and transverse colon, or distal for the descending and sigmoid colon. The response to irinotecan was scored according to the WHO criteria.

\(^b\) Wild-type, both BAX alleles have a normal poly-G8 tract; Mutant, deletion or insertion of G in the poly-G8 tract; nd, not determined.

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biallelic size variations of BAT25 and/or BAT26 that were characteristic profile in MSI tumors. The observed sizes were shortened by 5–10 bp for BAT25 and 6–12 bp for BAT26, which is far outside the range of normal allelic sizes. In four cases (#17, #34, #46, and #150), both BAT25 and BAT26 were unstable, whereas two tumors (#43 and #115) displayed instability at the BAT26 locus, with a normal BAT25 profile. In tumor #37, BAT26 could not be amplified, whereas BAT25 was stable. Using the complete Bethesda panel, this tumor, like the tumors that displayed instability in BAT25 and/or BAT26, could be unambiguously classified as MSI-H because at least two additional markers displayed instability (Table 3). Among the 44 primary colorectal tumors, 5 (11.4%) were MSI-H, whereas 2 of the 28 (7.1%) metastatic lesions analyzed displayed high MSI, indicating that in our series, the incidence of MSI tumors did not differ significantly between primary colorectal tumors and metastases (P = 0.7, nonsignificant). Among these 7 MSI-H tumors, 5 arose in the proximal colon and 2 in the distal colon; no cases of rectal cancer were found in this group. Four (57.1%) of these MSI-H tumors partially regressed on treatment with IRI, whereas 1 was stabilized, and 2 progressed. Among the 65 MSI-L/microsatellite stable tumors, 7 (10.8%) tumors responded to IRI, 28 were stabilized, and 30 continued to progress under treatment. The relationship between the MSI phenotype and response to IRI chemotherapy is statistically significant (P = 0.009; Table 4).

**Relationship between Mutations in cMNRs and the Response to IRI.** We looked for frameshift mutations in the cMNRs contained in four genes, TGFβ-RII, BAX, hMSH3, and hMSH6 (Table 3). We analyzed the TGFβ-RII poly-A10 microsatellite and found three primary tumors displaying a frameshift mutation because of a 1-bp deletion (#17, #46, and #150). All three tumors with mutations in TGFβ-RII exhibited high levels of instability at the Bethesda markers and occurred in the proximal colon. Two of them (#17 and #46) partially regressed on treatment with IRI; one (#17) of these tumors also carried a 1-bp deletion in the poly-A8 tract of the hMSH3 gene. The third tumor carrying a mutation in TGFβ-RII (#150) also displayed a 1-bp insertion in the poly-C8 of hMsh6 and a 1-bp deletion in the poly-G8 repeat of BAX and remained stable on treatment (Table 3). Three unrelated tumors (#31, #34, and #37) displayed a 1-bp deletion in the poly-G8 tract of BAX, which resulted in the extinction of BAX expression as assessed by IHC. No other mutation in the target genes studied could be found in these three BAX-negative tumors. Interestingly, all three these cases corresponded to hepatic metastases and partially responded to IRI. Using the Bethesda panel, two of them (#34 and #37) could be classified MSI-H, whereas the third tumor (#31) was MSI-L. In one of the BAX-mutated MSI-H tumors (#34), the expression of hMSH2 was undetectable by IHC. Two tumors with mutations in hMSH6 could be detected in our series (#115 and #150); both these mutations resulted from a 1-bp addition in the poly-C8 tract and occurred in MSI-H tumors. One of them (#150) also had a 1-bp deletion in both TGFβ-RII and BAX genes, whereas the other had a 1-bp deletion in MSH3 (#115).

Interestingly, 5 of the 7 (71.4%) tumors displaying inactivation of TGFβ-RII, BAX, or the hMSH3 gene responded to IRI, versus 6 of the 65 (9.2%) tumors without any mutation in the cMNRs of these genes (P < 0.001; Table 4).

### DISCUSSION

Numerous studies have reported that tumors with or without MSI display different clinicopathological features. Indeed, MSI CRC are more likely to be of high histological grade, located in the proximal colon and associated with improved overall survival (33, 34). There is increasing evidence of a relation between the MMR status of tumor cells and their response to various chemotherapeutic drugs (reviewed in Refs. 35 and 36). In particular, *in vitro* studies have shown that MMR-deficient cell lines display moderate levels of resistance to methylation agents and low level resistance to cisplatin (37, 38). More recently, the hMLH1-deficient HCT116 CRC cell line, a prototype of MSI cell lines, was found to be slightly more resistant to the cytotoxicity of 5-FU (39, 40). Unfortunately, the few clinical studies that have been performed addressing this issue have come to contradictory conclusions. A recent study reported survival benefits in patients with MSI tumors who received adjuvant treatment with 5-FU, but these results have been challenged (41, 42). Yet, the analysis of survival benefit in a similar group of patients led these authors to opposite findings (41).

To define molecular criteria predictive for tumor responsiveness to chemotherapy, we have investigated previously the role of MMR in a panel of MMR-defective colorectal cell lines and shown that a defect in MMR results in increased sensitivity to CPT (6). Although neither p53 status nor endogenous topoisomerase I levels could predict the cellular sensitivity to CPT, we could establish that MMR status of the cells is a critical determinant for chemosensitivity of colorectal cell lines (6). In addition, using a model of CRC xenografts in nude mice, it has also been shown that the MSI phenotype moderately increases sensitivity to IRI (43). This is in agreement with our preliminary data.

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**Table 3 Relationship between MSI phenotype, frameshift mutation in cMNR of target genes, and the response of metastatic colorectal cancer to the treatment with irinotecan**

<table>
<thead>
<tr>
<th>Case</th>
<th>Primary tumor site</th>
<th>Tumor specimen studied</th>
<th>Irinotecan tumor response</th>
<th>MSI phenotype</th>
<th>TGFβ-RII</th>
<th>BAX</th>
<th>hMSH3</th>
<th>hMSH6</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Proximal</td>
<td>Primary tumor</td>
<td>Partial regression</td>
<td>High</td>
<td>mut&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—&lt;sup&gt;b&lt;/sup&gt;</td>
<td>mut</td>
<td>—</td>
</tr>
<tr>
<td>31</td>
<td>Distal</td>
<td>Metastasis</td>
<td>Partial regression</td>
<td>Low</td>
<td>—&lt;sup&gt;c&lt;/sup&gt;</td>
<td>mut</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>34</td>
<td>Proximal</td>
<td>Metastasis</td>
<td>Partial regression</td>
<td>High</td>
<td>—&lt;sup&gt;c&lt;/sup&gt;</td>
<td>mut</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>37</td>
<td>Distal</td>
<td>Metastasis</td>
<td>Partial regression</td>
<td>High</td>
<td>—&lt;sup&gt;c&lt;/sup&gt;</td>
<td>mut</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>43</td>
<td>Distal</td>
<td>Primary tumor</td>
<td>Progression</td>
<td>High</td>
<td>—&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>46</td>
<td>Proximal</td>
<td>Primary tumor</td>
<td>Partial regression</td>
<td>High</td>
<td>mut</td>
<td>—</td>
<td>mut</td>
<td>mut</td>
</tr>
<tr>
<td>115</td>
<td>Proximal</td>
<td>Primary tumor</td>
<td>Progression</td>
<td>High</td>
<td>—&lt;sup&gt;c&lt;/sup&gt;</td>
<td>mut</td>
<td>mut</td>
<td>mut</td>
</tr>
<tr>
<td>150</td>
<td>Proximal</td>
<td>Primary tumor</td>
<td>Stabilization</td>
<td>High</td>
<td>mut</td>
<td>mut</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a</sup> The primary tumor site is proximal for tumor originating in the ascending and transverse colon or distal for the descending and sigmoid colon. The response to irinotecan was scored according to the WHO guidelines. The MSI phenotype is defined according to the Bethesda recommendations.

<sup>b</sup> mut, presence of a mutation in the target gene mononucleotide repeats.

<sup>c</sup> —, no mutation.

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**Table 4 Relationship between the response of patients with advanced colorectal cancer to chemotherapy with irinotecan and tumor alterations**

<table>
<thead>
<tr>
<th>Response to IRI</th>
<th>BAX&lt;sup&gt;mut&lt;/sup&gt;</th>
<th>BAX&lt;sup&gt;wt&lt;/sup&gt;</th>
<th>MSI-L/MSS</th>
<th>MSI-H</th>
<th>MSI-L/MSS&lt;sup&gt;mut&lt;/sup&gt;</th>
<th>cMNR-MSI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responder</td>
<td>3</td>
<td>8</td>
<td>4</td>
<td>7</td>
<td>5</td>
<td>6</td>
<td>0.01</td>
</tr>
<tr>
<td>Nonresponder</td>
<td>1</td>
<td>60</td>
<td>3</td>
<td>58</td>
<td>2</td>
<td>59</td>
<td>0.009</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Response to IRI</th>
<th>BAX&lt;sup&gt;mut&lt;/sup&gt;</th>
<th>BAX&lt;sup&gt;wt&lt;/sup&gt;</th>
<th>MSI-L/MSS</th>
<th>MSI-H</th>
<th>MSI-L/MSS&lt;sup&gt;mut&lt;/sup&gt;</th>
<th>cMNR-MSI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responder</td>
<td>3</td>
<td>8</td>
<td>4</td>
<td>7</td>
<td>5</td>
<td>6</td>
<td>0.01</td>
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<tr>
<td>Nonresponder</td>
<td>1</td>
<td>60</td>
<td>3</td>
<td>58</td>
<td>2</td>
<td>59</td>
<td>0.009</td>
</tr>
</tbody>
</table>

<sup>a</sup> The associations between the tumor response to irinotecan and the mutations in the BAX-polyG8 tract, the MSI, or cMNR-MSI phenotypes were assessed using Fisher’s exact test.

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of hMLH1 expression, confirming that in the absence of their partner, tumors, whereas loss of hPMS2 expression was concomitant with loss of hMSH6 was mainly observed in hMSH2-negative tumors only or hPMS2 only occurred in rare instances (47). Abnormal expression of hMSH6 or hPMS2, it is rather unlikely that a defect in these genes accounts for the MSI phenotype. Yet, in a recent study that assessed the expression pattern of all four MMR proteins in a series of MSI tumors, it was reported that loss of hMSH6 only or hPMS2 only occurred in rare instances (47). Abnormal expression of hMSH6 was mainly observed in hMSH2-negative tumors, whereas loss of hPMS2 expression was concomitant with loss of hMLH1 expression, confirming that in the absence of their partner, these proteins are unstable (47). Given that hMLH1 inactivation is responsible for the majority of MSI sporadic CRC, this phenomenon is expected to occur in ~10–15% of unselected primary CRC (23, 24, 48). Whether the occurrence of hMLH1 inactivation in metastatic CRC is comparable has not yet been reported. Yet the loss of hMLH1 expression appears to be less frequent among the 5-FU-resistant metastatic CRC cases that we have analyzed. Several explanations may account for this observation: (a) cells that had lost hMLH1 expression in the primary tumor may have been counter-selected on the treatment with 5-FU; (b) the selection of tumor cells with high metastatic potential that occurs during tumor progression may apply to cells expressing hMLH1; and (c) several drugs are known to induce demethylation of DNA resulting in the re-expression of genes when silencing is caused by promoter hypermethylation. In particular, this phenomenon has been documented for hMLH1 both in vitro experiments and in a model of human colorectal tumor xenografts (24, 49). Thus, it is conceivable that re-expression of hMLH1 has taken place in a subset of tumor cells, particularly when patients are treated with DNA-damaging drugs.

We have determined the MSI phenotype using both BAT26 and BAT25 microsatellites, because their sensitivity and specificity are very similar to those of the Bethesda panel, allowing to establish the tumor MSI status with >99% accuracy, with no need for normal matched DNA (31, 32, 34, 50, 51). Interestingly, because BAT25 and BAT26 are mononucleotide repeats, they display instability not only in tumors with a defect in either hMSH2 or hMLH1 but also in hMSH6-deficient tumors (32, 52, 53). Given that we had observed that colorectal cells with a defect in hMSH6 also displayed increased sensitivity to CPT, we decided to use both BAT25 and BAT26 as phenotypical markers of the MSI phenotype to efficiently screen tumors whose MSI was restricted to mononucleotide repeats (6). We have further performed MSI analysis using the Bethesda panel on the cases that displayed instability at either BAT25 or BAT26 mononucleotide repeats or in the coding repeats. As expected, we have observed that tumors displaying MSI at BAT26 and/or BAT25 loci were MSI-H tumors, confirming that the use of BAT26 and BAT25 markers allows unambiguous identification of MSI tumors. It is worth noting that the rate of MSI tumors in our series of metastatic CRC is within the range reported for selected familial and sporadic CRC, challenging the idea that MSI colorectal tumors have a reduced risk of liver metastasis (54–56).

We further investigated the presence of inactivating mutations in coding repeats of genes whose role in colorectal carcinogenesis had been suspected. These included TGFβ-RII, a potent inhibitor of cell growth and tumor progression, BAX, a proapoptotic member of the Bcl-2 family, as well as hMSH3 and hMSH6, two DNA MMR components (28, 57, 58). Seven tumors displayed a mutation in at least one of the genes analyzed. Three primary CRC tumors displayed a frameshift mutation in the TGFβ-RII gene. The inactivation of the TGFβ-RII gene has been reported in 70–90% of MSI CRC and is believed to occur at an early stage, during the transition from colon adenoma to carcinoma (30, 59–62). In our series, the TGFβ-RII mutations are underrepresented and restricted to primary tumors, indicating that they may be counter-selected during the metastatic process. Conversely, all MSI-driven mutations of BAX were observed in metastatic lesions. Although the inactivation of the BAX gene occurs in approximately half of the MSI primary CRC, data concerning hepatic metastasis are not available (58, 60, 63). BAX inactivation is apparently not required for the initiation step of the tumorigenic process but rather confers a selective advantage during clonal evolution (60, 64). It is remarkable that the expression level of BAX is significantly lower in the metastases compared with the primary colorectal tumors (65). The tumors with the lowest expression of BAX displayed a more infiltrative growth pattern and more distal metasteses (65). In this context, our results showing that BAX inactivation was predominantly observed in hepatic metastases indicate a possible role of BAX in the metastatic progression of CRC.

The molecular mechanisms underlying the hypersensitivity of MSI tumors to IRI are not yet clear. In most tumors, the MSI phenotype results from the inactivation of either hMSH2 or hMLH1, two components of MMR that have been shown to participate in recombination. Given that IRI acts by generating DSB in DNA, a decrease in recombinational repair efficiency resulting from a defect in MMR could account for the higher chemosensitivity of MSI tumors, a hypothesis that is currently under investigation in our laboratory. Moreover, a link between MMR deficiency and loss of normal cell cycle control, particularly G2 arrest, has been established. Because DSBs are lethal lesions if not repaired before mitosis, a defect in G2 checkpoint in response to IRI-induced damage may also contribute to increase its cytotoxicity. In addition, any gene that contains a microsatellite repeat is a potential target for MSI-driven insertion/deletion mutations. Consequently, MSI tumors accumulate widespread mutations, not only in genes that participate in tumor initiation and progression but also in genes that are involved in various DNA repair pathways, e.g., several reports have shown that both MRE11 and RAD50 are frequently inactivated in MSI tumors (66, 67). These genes being part of the MRE11-NBS1-RAD50 complex, which plays a key role in DSB repair, it is reasonable to speculate that their defect contributes to enhance IRI-induced cytotoxicity. Other DNA repair genes contain coding microsatellite coding repeats and may therefore be inactivated in MSI tumors. It follows that the sensitivity of MSI CRC to IRI may not be a direct consequence of MMR deficiency itself but may rather be the result of the impairment of a crucial DNA repair pathway.

In conclusion, this study allowed us to establish that the MSI phenotype and loss of BAX expression are thus far the best criteria for selecting patients who could benefit from chemotherapy with IRI. Therefore, provided that these results are confirmed on a larger series, MSI phenotyping should be routinely performed to improve the clinical management of patients with CRC.
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REFERENCES


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