Molecular Pathways: Microsatellite Instability in Colorectal Cancer: Prognostic, Predictive, and Therapeutic Implications

Frank A. Sinicrope1,2 and Daniel J. Sargent3

Abstract

Microsatellite instability (MSI) is the molecular fingerprint of the deficient mismatch repair (MMR) system, which characterizes ~15% of colorectal cancers. MSI develops as a result of germline mutations in MMR genes or, more commonly, from epigenetic silencing of MLH1 in sporadic tumors occurring in a background of methylation of CpG islands in gene promoter regions and in tumors that frequently show hotspot mutations in the BRAF oncogene. MSI tumors have distinct phenotypic features and have been consistently associated with a better stage-adjusted prognosis compared with microsatellite stable tumors. MSI negatively predicts response to 5-fluorouracil and may also determine responsiveness to other drugs used for treatment of colorectal cancers. Recent data have expanded the molecular heterogeneity of MSI tumors and may contribute to our understanding of differential chemosensitivity. The ability to identify deficient MMR has important implications for patient management, and it holds promise for therapeutic exploitation and for the development of novel therapeutics.

Background

The future of personalized oncology relies on the identification of molecular subtypes of cancer for which targeted therapeutic agents can be developed or selectively utilized. Investigators have identified 2 molecularly distinct pathways of colorectal tumorigenesis: chromosomal instability and microsatellite instability [MSI (1)]. The majority of colorectal cancers show chromosome instability characterized by loss or gain of chromosome arms, chromosomal translocations, or gene amplifications (1). In contrast, inactivation of a DNA mismatch repair (MMR) gene (MLH1, MSH2, MSH6, or PMS2) by mutation or transcriptional silencing results in deficient function of the MMR system and an accumulation of errors in DNA within microsatellites that is termed MSI (2, 3). Microsatellites are short, repetitive DNA sequences that are found throughout the tumor genome and are prone to mutations. The most frequent errors associated with microsatellites are base-base mismatches, and insertions/deletions in DNA coding regions produce frameshift mutations that can lead to protein truncations. The MMR system functions to correct errors introduced in microsatellites through a series of steps involving the interaction of MMR proteins as heterodimers with a complex formed by a MutS and a MutL (Fig. 1; ref. 4).

When a mismatch is detected, MSH2 associates with either MSH6 or MSH3 (5) to form a MutSα or MutSβ complex, respectively. Furthermore, MLH1 interacts with PMS2, PMS1, or MLH3 to form a MutLα, MutLβ, or MutLγ complex, respectively (4). Excision of the mismatch is performed by proteins such as exonuclease 1 and proliferating cell nuclear antigen (PCNA), and it is followed by resynthesis and religation of the DNA strand (4). In addition to MMR, DNA repair systems also include base excision repair, which removes small, non–helix-distorting base lesions from the genome, and the related nucleotide excision repair pathway, which repairs bulky, helix-distorting lesions. PARP has emerged as an important therapeutic target because its inhibition leads to blockage of base excision repair and is especially toxic in BRCA-deficient tumor cells that are more dependent on PARP and homologous recombination to repair DNA damage (6). Nuclear PARP1 is activated by DNA strand breaks and is involved in DNA repair.

MSI tumors carry mutations in mononucleotide tracts in the coding regions of several genes, including BRAFV600E, TGIFIRII, BAX, and IGFIRI (1). Deficient MMR and MSI arise due to germline mutations in MMR genes or, more commonly, from somatic hypermethylation of CpG islands surrounding the promoter region of MLH1 and other genes, which is known as the CpG island methylator phenotype (CIMP [Fig. 2; refs. 7, 8]). CIMP tumors compose the majority of sporadic MSI colorectal cancers (9). In addition to epigenetic inactivation of MLH1, Nagasaka and colleagues (10) reported a heritable somatic methylation of MSH2 that is caused by a deletion of the last exon of EPCAM, which is adjacent to MSH2. Germline MMR mutations give rise to Lynch syndrome (also known as hereditary nonpolyposis colorectal cancer), an autosomal dominant disorder that accounts for ~3% of all colorectal cancers...
Deficient MMR is indicated by MSI or loss of an MMR protein. Although investigators routinely perform MSI testing and/or analysis of MMR protein expression by immunohistochemistry to identify patients with suspected Lynch syndrome (11), the use of these approaches outside of this indication has yet to gain widespread acceptance.

Colorectal cancers with MSI have distinct pathologic features, such as proximal colon predominance, poor differentiation and/or mucinous histology, and increased numbers of tumor-infiltrating lymphocytes (12, 13). In population-based studies, the prevalence of MSI among colorectal cancers is ~15% (9, 14) and is more common among stage II compared with lymph node–positive or stage III colorectal cancers. MSI is relatively uncommon among stage IV or metastatic colorectal cancers (~4% (15)). Among colorectal cancers, MSI is detected more frequently in women, especially older women, compared with men given differences in MLH1 methylation frequencies (9). Both observational studies and data from patients enrolled in randomized clinical trials have consistently shown that deficient MMR is independently associated with improved survival compared with tumors showing proficient MMR that includes microsatellite stable (MSS) tumors (13, 14, 16). These findings are further supported by the results of a meta-analysis (17). Patients with deficient

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Figure 1. The MMR system functions to correct errors introduced in microsatellites through a series of steps involving the interaction of MMR proteins as heterodimers. A, MSH2-MSH6 (MutSα) recognizes single base-pair mismatches, shown by the incorrect base (G) matched with T on the template, and creates a sliding clamp around the DNA. This step requires the exchange of ATP for ADP. The MutSα complex is then bound by the MLH1-PMS2 (MutLα) complex. B, excision of the mismatch occurs when the DNA MMR protein sliding clamp interacts with exonuclease-1, PCNA, and DNA polymerase. This complex excises the daughter strand back to the site of the mismatch. The complex comes off the DNA, and resynthesis then occurs with correction of the error. C, MSH2-MSH3 (MutSβ) can recognize larger insertion/deletion loops that can complement the function of MSH2-MSH6, which recognizes single pair mismatches and small insertion/deletion loops. Potential interactions with other MutL dimers are shown because MLH1 can dimerize with PMS2, PMS1, or MLH3. [Reprinted with permission from Elsevier (ref. 4).]
MMR tumors also have significantly reduced rates of tumor recurrence compared with those with proficient MMR tumors (18, 19). MSI colorectal cancers typically have diploid DNA content, and in a previous study (20) the prognostic impact of deficient MMR was no longer evident when ploidy was taken into account. Investigators recently reported confirmatory prognostic data from large clinical trials [i.e., the Quick and Simple and Reliable (QUASAR) trial (19) and Pan-European Trials in Alimentary Tract Cancers 3 (PETACC-3; refs. 21, 22)] in patients with stage II and/or III colon carcinomas. Despite the aforementioned data, testing for MSI or MMR proteins has not been routinely incorporated into clinical practice to inform patients about their prognosis or to guide management. To address the lack of prospective data, an ongoing trial in stage II colon cancers is categorizing patients into high- and low-risk groups based on MSI status and allelic loss at 18q (ECOG-E5202). Patients with low-risk tumors, defined as having MSI and intact 18q, are assigned to no postoperative treatment, whereas high-risk patients receive the standard adjuvant regimen for stage III colorectal cancer, which is 5-fluorouracil (5-FU) and oxaliplatin. Because the MSI tumors are not treated with 5-FU, the study will not provide predictive information regarding MSI and 5-FU–based treatment.

Clinical–Translational Advances

Emerging evidence suggests that certain microRNAs (miRNA) can regulate MMR expression to influence genomic stability in colorectal cancer. Ectopic expression of miR-155 or miR-21 was independently shown to downregulate MMR proteins and to induce MSI in colorectal cancer cells (3, 24). In human colorectal cancers, overexpression of miR-155 or miR-21 was inversely related to the level of hMLH1 and/or hMSH2 protein expression (23, 24). Furthermore, miR-155 overexpression was found in a tumor subset with an unknown cause of MMR inactivation (23). In a colorectal cancer xenograft model, miR-21 overexpression was shown to markedly reduce the efficacy of 5-FU, and this was associated with downregulation of hMSH2 (24). Together, these preliminary data suggest a potential role for these miRNAs in the pathogenesis of MSI and as a potential indicator of 5-FU response.

Recent data expand the molecular heterogeneity found within MSI colorectal cancers. A mutation in the gene encoding heat shock protein (HSP) 110 was found in MSI cell lines and human colorectal cancers (25). The HSP110 truncated protein lacked chaperone activities or antiapoptotic properties typical of HSPs. Although very preliminary, this mutation was shown to sensitize MSI colorectal cancer cells to treatment with 5-FU and oxaliplatin, and there was a suggestion of survival benefit in 2 small retrospective cohorts of MSI colorectal cancers treated with adjuvant 5-FU with or without oxaliplatin (25). In previous studies, MSI tumors were associated with higher rates of inactivation of the PTEN tumor suppressor gene by mutation or hypermethylation compared with MSS tumors (26, 27).

Sporadic MSI colon cancers with epigenetic inactivation of MLH1 show frequent (~50%) co-occurrence of BRAFV600E mutations compared with an overall BRAF...
mutation frequency of 8% to 11% among colorectal cancers (21, 28). BRAF encodes a serine/threonine kinase that is an essential component of the RAF/MEK/ERK signaling cascade (29, 30). In colorectal cancers, BRAF mutations are located in a hotspot in exon 15 that leads to a V600E single-amino-acid substitution (29). BRAF mutations are mutually exclusive with KRAS mutations that are more commonly associated with MSS tumors (30). The presence of a BRAF mutation indicates a sporadic MSI colorectal cancer and essentially excludes a diagnosis of Lynch syndrome (31). Within colorectal cancers, BRAFV600E mutations have been associated with a worse prognosis across tumor stages (28, 32). However, conflicting data in stage II/III colon cancer patients participating in adjuvant chemotherapy trials were recently reported. Specifically, BRAF mutation was not prognostic in stage II tumors in the QUASAR adjuvant study (19), but it was associated with reduced overall survival (but not recurrence-free survival) in stage II/III tumors in the PETACC-3 adjuvant trial (22). It remains to be determined whether BRAF mutations can confer prognostic information within the subgroup of MSI tumors (33).

Limited data are available with regard to the prognostic or predictive impact of CIMP. Findings from a large study suggest that CIMP-high is associated with a favorable prognosis in colorectal cancer patients, independently of MSI and BRAF mutation status (32). Studies that examined the predictive utility of CIMP for 5-FU–based therapy were inconclusive (34, 35). A recent analysis of a population-based cohort of patients with stage II and III colon cancers showed that patients with CIMP-positive tumors did not benefit from adjuvant 5-FU, whereas patients with CIMP-negative tumors treated with 5-FU showed improved survival (36). Of importance, the discrepancies among these studies may be related to the different methylation markers and definitions of CIMP used (8, 32, 37). Another unresolved issue is whether germline tumors and MSI tumors of sporadic origin have different chemosensitivities. In a recent study, tumor metastases were reduced by 5-FU–based adjuvant treatment in stage III colon cancers with deficient or proficient MMR, and a subset analysis of deficient MMR cases suggested that any treatment benefit was restricted to suspected germline tumors (18). Although provocative, these data await confirmation in an independent dataset that includes genotyping for MMR genes.

Studies have shown that MSI is a negative predictive marker of response to 5-FU. In a study using MLH1-deficient HCT116 colon cancer cells that displayed resistance to 5-FU, transfer of chromosome 3 was shown to introduce a functional MLH1 gene that was shown to restore sensitivity to 5-FU (38). Similarly, reexpression of hMLH1 by the demethylating drug 5-azacytidine was shown to restore 5-FU sensitivity (39). Tumor xenograft studies confirmed that MSI is associated with resistance to 5-FU (40). Most, but not all (41, 42), studies of patients with MSI tumors showed a lack of survival benefit from 5-FU as an adjuvant treatment for colon cancer. These studies included randomized clinical trials (13, 16), retrospective case series (43, 44), and a meta-analysis (17). Relevant to this issue are data from colorectal cancers showing that MSI status and thymidylate synthase expression are unrelated (18). Ideally, predictive marker analyses are conducted in studies that incorporate untreated control arms. Although most studies do not meet this criterion, data from stage II/III colon cancer patients treated with 5-FU–based adjuvant therapy versus observation confirmed a lack of benefit for 5-FU in deficient MMR tumors compared with proficient MMR tumors (16). Accordingly, it was recommended that patients with stage II colon cancer showing MSI should not receive 5-FU as adjuvant therapy, given their favorable prognosis and lack of benefit from 5-FU (16, 45).

Preclinical studies suggest that MSI colon cancer cells and tumor xenografts are more sensitive to irinotecan compared with MSS cells (46–48); however, the molecular mechanisms are only partially defined. Irinotecan is a camptothecin analogue and a potent inhibitor of the topoisomerase 1 enzyme that results in the inhibition of DNA replication. MSI colorectal cancer cell lines and human tumors carry frequent mutations in the MRE11A and hRAD50 genes that control repair of DNA double-strand breaks (46). Mutations in these genes were shown to confer greater sensitivity to camptothecins compared with cells with wild-type copies (46). These data suggest that secondary mutations in genes that regulate DNA double-strand breaks, rather than MSI itself, may be responsible for increased sensitivity to camptothecins. Of note, MRE11A mutations are found in 70% to 85% of MSI colon cancers (49, 50). The utility of MSI for predicting the efficacy of irinotecan has been studied in patients with colon cancer. In an adjuvant trial (CALGB 89803), a statistically significant improvement in disease-free survival was seen in MSI compared with MSS tumors upon addition of irinotecan to 5-FU/leucovorin (51). However, this finding was not supported by another adjuvant trial (PETACC-3) in patients with stage II and III colon cancer (21). Investigators in this trial compared infusional 5-FU/leucovorin with or without irinotecan and found that the addition of irinotecan did not improve survival in patients with MSI versus MSS tumors. Therefore, the issue of an irinotecan benefit in MSI colon cancers is unresolved and requires further study.

Studies have shown that MMR-deficient cells are resistant to cisplatin and carboplatin, in contrast with oxaliplatin. MMR proteins do not recognize oxaliplatin-related adducts because oxaliplatin contains a bulky moiety that becomes incorporated into DNA via cytotoxic intra- and interstrand adducts (52). Accordingly, oxaliplatin chemosensitivity is independent of the MMR system (53). Recent data indicate that the MSH3 MMR gene confers resistance to oxaliplatin and cisplatin, and its suppression can restore sensitivity to these drugs (54). MSH3 and MSH2 form the MutSβ heteroduplex, which interacts with interstrand cross-links induced by platinum-based anticancer drugs (54). The MSH3 gene frequently undergoes somatic mutation in MMR-deficient colorectal cancers (55, 56). To date, only limited data are available regarding MSI tumors in patients treated with oxaliplatin combined with 5-FU, and predictive
information is lacking. A retrospective single-arm study in 5-FU-plus-oxaliplatin-treated patients with stage III colon cancer found that the 3-year disease-free survival rate was significantly higher in patients with deficient MMR tumors compared with proficient MMR tumors (57). This suggests that the prognostic impact of MMR status is maintained because oxaliplatin is expected to provide an equivalent benefit irrespective of MMR status.

In addition to using MMR status to guide treatment decisions, an important goal is to develop molecularly targeted agents to exploit the specific mechanisms of MSI cancers. Evaluation of the predictive impact of MSI is limited by its 4% prevalence in metastatic colorectal cancers (15). Data suggest the utility of PARP inhibitors in MSI tumors deficient in homologous recombination due to mutations in the coding microsatellites of the MRE11A and hRAD50 genes involved in double-strand DNA repair (50). Preferential cytotoxicity to the PARP-1 inhibitor ABT-888 was seen in MSI cell lines containing mutant copies of MRE11A compared with wild-type or MSS cells (58). In a recent study, the observed ability of MSH3 to protect against double-strand breaks was exploited by the combination of oxaliplatin and a PARP inhibitor, which produced a synergistic cytotoxic effect against colorectal cancer cells (54). These data suggest that synthetic lethality can potentially be exploited in MSI cancers. A screen to identify drugs that induce death in MSH2-deficient tumor cells identified methotrexate, which is supported by the finding that suppression of dihydrofolate reductase led to increased death in these same cells (59).

An ongoing, single-arm, phase II study is evaluating methotrexate in MSH2-deficient advanced colorectal cancers. Using high-throughput array technology for molecular profiling of tumor tissue, Vilar and colleagues (60) identified drugs that target the PI3K/AKT/mTOR pathway and showed that they selectively inhibit MSI cancer cell lines. These data suggest the relevance of targeting this pathway in the MSI tumor subtype. Given that chromosomal instability is associated with taxane resistance, it has been hypothesized that MSI tumors may exhibit greater sensitivity to taxane therapy. To test this hypothesis, a clinical trial is currently evaluating patupilone, a microtubule-stabilizing drug, in patients with MSI tumors (61). Another potential strategy is to use demethylating agents (e.g., decitabine), which can demethylate MLH1 but lacks selectivity. Given that BRAF mutations are common in sporadic MSI colorectal cancers (31, 32), selective inhibitors of BRAF, such as PLX-4032, are of therapeutic interest in these tumors. Because deficient MMR represents a relatively small molecular subtype, multi-institutional studies will be needed to recruit sufficient numbers of patients for adequately powered clinical trials. Going forward, RNA interference screening studies may identify new genetic targets within MSI tumors that are important for drug development.

Conclusions

The recognition of molecular subtypes of human cancers represents the future of personalized oncology and will guide drug-development strategies. colorectal cancers with MSI have distinct phenotypic features and a molecular etiology that includes epigenetic inactivation of MLH1 and somatic BRAF mutations versus germline mutations in MMR genes conferring Lynch syndrome. There is increasing evidence of further molecular heterogeneity in MSI tumors with respect to secondary mutations in genes that regulate diverse biologic processes. Evidence indicates that MSI tumors exhibit differences in their clinical behavior and response to anticancer drugs compared with MSS cancers. MSI tumors have been consistently associated with a favorable prognosis and an apparent resistance to treatment with 5-FU, although the predictive impact of MMR for oxaliplatin or irinotecan in MSI tumors awaits further study. The interpretation of these data from patient studies is complicated by the combination of drugs with 5-FU. Well-designed clinical trials based on molecular classification are needed to resolve treatment-response issues. Given the increasing evidence of the molecular heterogeneity of MSI tumors, strategies are needed to exploit the specific mechanisms of MSI cancers for therapeutic advantage. Furthermore, the use of high-throughput technology for molecular profiling and screening for novel drug targets holds promise for the development of novel therapeutic strategies.

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