

Microsatellite instability in colorectal cancer—the stable evidence

Eduardo Vilar and Stephen B. Gruber

Abstract | Microsatellite instability (MSI) is the molecular fingerprint of a deficient mismatch repair system. Approximately 15% of colorectal cancers (CRC) display MSI owing either to epigenetic silencing of *MLH1* or a germline mutation in one of the mismatch repair genes *MLH1*, *MSH2*, *MSH6* or *PMS2*. Methods to detect MSI are well established and routinely incorporated into clinical practice. A clinical and molecular profile of MSI tumors has been described, leading to the concept of an MSI phenotype in CRC. Studies have confirmed that MSI tumors have a better prognosis than microsatellite stable CRC, but MSI cancers do not necessarily have the same response to the chemotherapeutic strategies used to treat microsatellite stable tumors. Specifically, stage II MSI tumors might not benefit from 5-fluorouracil-based adjuvant chemotherapy regimens. New data suggest possible advantages of irinotecan-based regimens, but these findings require further clarification. Characterization of the molecular basis of MSI in CRC is underway and initial results show that mutations in genes encoding kinases and candidate genes with microsatellite tracts are over-represented in MSI tumors. Transcriptome expression profiles of MSI tumors and systems biology approaches are providing the opportunity to develop targeted therapeutics for MSI CRC.

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Introduction

In 1993, seminal articles reported the presence of microsatellite instability (MSI) as a frequent molecular phenomenon in colorectal cancers (CRC).^{1–3} Since then, a plethora of studies using various approaches have characterized this molecular subtype. Tumors harboring a deficient mismatch repair (MMR) system owing to germline, somatic or epigenetic inactivation account for approximately 15% of CRC diagnosed in the US.^{4,5} Although a large population of patients with CRC have a deficient MMR system, MSI has only slowly been recognized as a clinically relevant aspect of tumor biology even though it is a well established molecular marker for patients with suspected Lynch syndrome.⁶ This Review provides an introduction to the molecular basis of the MMR system, the detection of MSI, and the molecular characteristics of these tumors. In addition, it focuses on the clinical features of MSI tumors and the prognostic and predictive value of MSI. Finally, new targeted therapies for the treatment of this tumor subtype are discussed.

Molecular basis of the MMR system

Microsatellites are repetitive sequences distributed throughout the human genome that consist of mono-nucleotide, dinucleotide or higher-order nucleotide repeats such as (A)_n or (CA)_n. These sequence motifs are especially prone to accumulation of mutations, mainly because the DNA polymerases cannot bind DNA efficiently during DNA synthesis. The most frequent errors

associated with microsatellites are base–base mismatches that escape the intrinsic proofreading activity of DNA polymerases, and insertion–deletion loops, which are extrahelical nucleotides that form DNA hairpins. These unpaired nucleotides occur when the first nucleotide and template strand dissociate and incorrectly reanneal in a microsatellite. Insertions or deletions in microsatellites located in DNA coding regions generate frameshift mutations, which can lead to protein truncations.⁷

The MMR system is responsible for the surveillance and correction of errors introduced in microsatellites (Figure 1), and is highly conserved from bacteria to humans. *MLH1*, *MSH2*, *MSH3*, *MSH6* and *PMS2* are the main proteins involved in this system, and they interact as heterodimers. When a mismatch is detected, a number of steps take place: *MSH2* associates with either *MSH6* or *MSH3* (forming MutS α and MutS β complexes, respectively), and *MLH1* couples with *PMS2*, *PMS1* or *MLH3* (forming MutL α , MutL β or MutL γ complexes, respectively). The recognition of mismatches and insertion–deletion loops is carried out by the complex formed by a MutS and a MutL, which interacts with replication factor C. Excision of the mismatch is performed by proteins such as exonuclease 1 and proliferating-cell-nuclear antigen. Finally, resynthesis and religation of the DNA strand is carried out by DNA polymerase δ and DNA ligase.^{7,8} Mutations in the genes responsible for the recognition step lead to an accumulation of errors in DNA, which results in MSI.

MSI has been observed in diverse tumor types, including CRC, gastric, endometrial, ovarian and sebaceous

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Competing interests

The authors declare no competing interests.

Key points

- Microsatellite instability (MSI) is present in approximately 15% of colorectal cancers (CRC), which are mostly nonfamilial (sporadic) and caused by hypermethylation of the *MLH1* promoter
- Approximately 2–3% of all CRC are caused by germline mutations in one of the mismatch repair genes (*MLH1*, *MSH2*, *MSH6* and *PMS2*)
- The MSI phenotype is characterized by right-sided location, low pathological stage, mucinous presentation, tumor-infiltrating lymphocytes, absence of necrotic cellular debris and the presence of a Crohn-like nodular infiltrate
- MSI tumors have a good prognosis and reduced likelihood of metastasis compared with microsatellite stable tumors, which highlights the value of MSI as a prognostic marker in CRC
- Although 5-fluorouracil-based chemotherapy is the gold standard for CRC, this drug offers little benefit in early MSI CRC—irinotecan-based regimens and other drugs may hold promise, and are being studied in MSI CRC
- Transcriptome expression studies that characterize MSI tumors and cell lines have identified unique attributes of MSI cancers, and systems biology tools and other approaches enable investigation of targeted therapies

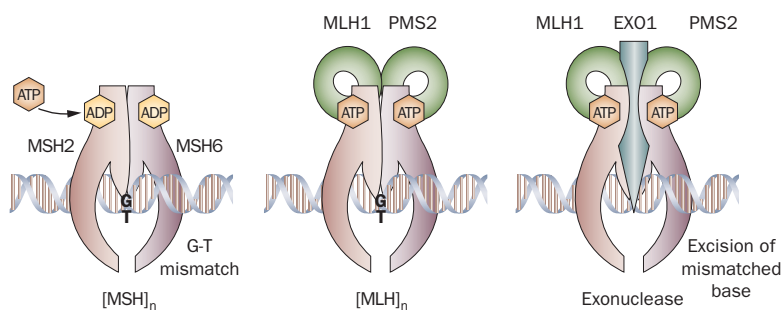


Figure 1 | Model of the proposed mechanism of mismatch repair proteins, illustrating patterns of clinically relevant heterodimerization. When a mismatch is detected, MSH2 associates with either MSH6 or MSH3, and MLH1 couples with PMS2, PMS1 or MLH3. The recognition of mismatches and insertion–deletion loops is carried out by a MutS and a MutL complex. Excision of the mismatch is performed by proteins such as exonuclease 1 and proliferating-cell-nuclear antigen. Resynthesis and religation of the DNA strand is carried out by DNA polymerase δ and DNA ligase.

carcinomas, as well as glioblastoma and lymphomas.^{9,10} First discovered in CRC, the MSI phenotype continues to serve as the hallmark of defective MMR since it was observed that germline mutations in *MLH1*, *MSH2*, *MSH6* and *PMS2* are responsible for a genetic predisposition to CRC known as Lynch syndrome or hereditary nonpolyposis CRC (HNPCC).^{6,11} Lynch syndrome is an autosomal dominant disease that accounts for 2–3% of total CRC.^{4,5,12} A heritable somatic methylation of *MSH2* has also been reported that is caused by a deletion of the last exon of *EPCAM*, which is adjacent to *MSH2*.¹³ If germline mutations are harbored in MMR genes, the cumulative risk of developing CRC is 60–70% in men, and 30–40% in women; the cumulative risk of developing endometrial cancer is 40–80%.^{5,14,15} Most cases of MSI CRC are a result of hypermethylation of *MLH1*.¹⁶ Several population-based studies have reported that the prevalence of MSI in CRC ranges from 8% to 20%, although these values might differ according to tumor stage distribution.^{5,13,17,18} MSI is more common

among stage II (~20%) than stage III (~12%) CRC,¹⁹ and is even less frequent among stage IV CRC (~4%).²⁰ In addition, two small retrospective studies suggest that MSI tumors could be more frequent in African American (20–45%) and Egyptian (37%) populations.^{21–23} Although the prevalence could vary between populations, it is clear that the MSI phenotype represents a clinically meaningful proportion of CRC.

Detection of MSI

MSI is detected by PCR amplification of specific microsatellite repeats. The presence of instability is determined by comparing the length of nucleotide repeats in tumor cells and normal cells. Normal DNA is typically extracted from adjacent normal mucosa. The analysis of amplified DNA was initially performed with denaturing acrylamide gels and radiolabeled primers; however, this process has been made easier with fluorescent primers and capillary electrophoresis (Figure 2).^{24,25} In the late 1990s, a consensus conference established a panel of microsatellite markers with appropriate sensitivity and specificity to diagnose MSI CRC. This reference panel, known as the Bethesda panel, included five microsatellite loci: two mononucleotides (BAT25 and BAT26) and three dinucleotides (D5S346, D2S123 and D17S250). Some clinical and research laboratories have expanded this panel to ten markers, which has made the diagnosis of MSI CRC easier.^{9,26} Three categories of MSI have been established based on the following criteria: MSI-High (MSI-H), indicating instability at two or more loci (or >30% of loci if a larger panel of markers is used); MSI-Low (MSI-L), indicating instability at one locus (or in 10–30% of loci in larger panels), and microsatellite stable (MSS), indicating no loci with instability (or <10% of loci in larger panels). MSI-L cases usually only show instability for dinucleotide markers, so the assessment of dinucleotides alone could lead to the misclassification of MSS or MSI-L as MSI-H. By contrast, mononucleotides BAT25 and BAT26 are nearly monomorphic, so in the absence of normal control tissue, MSI determination could be based on the presence of mononucleotides for most non-African populations.²⁵ The Bethesda panel, therefore, has a sufficient combination of markers for MSI. Newer commercial kits include a predominance of mononucleotide markers with improved sensitivity.

Immunohistochemical analysis of MMR proteins has become a popular alternative to detect MSI in the clinical setting and as a complement to the genetic testing of Lynch syndrome. Antibodies against MLH1, MSH2, MSH6 and PMS2 provide insight into the functionality of the MMR system. Lack of expression of one or more of these proteins is diagnostic of deficient MMR, and determines which encoding gene is most likely to harbor a germline mutation or to have been inactivated by another mechanism. Interpretation of the immunohistochemical pattern takes advantage of the dependent expression of specific heterodimers in the molecular diagnostic workup of CRC, as illustrated in Table 1.⁶ As an example, CRC that lack expression of MLH1 and PMS2, but retain expression of MSH2 and MSH6,

represent deficient MLH1 expression (Figure 3). In this situation, absent expression of PMS2 is simply a consequence of the defective MLH1. Whether the deficiency of MLH1 is caused by inactivation of the gene by promoter hypermethylation or a germline mutation that causes Lynch syndrome requires further investigation, but immunohistochemistry directs the workup to focus on MLH1 rather than the other MMR proteins.

Molecular features of MSI tumors

The MSI phenotype is strongly associated with mutations in specific oncogenes and tumor suppressor genes, especially *BRAF* and *MRE11A*, and also in others, such as *KRAS*. The majority of CRC is characterized by genetic instability that arises through loss or gain of chromosome arms, chromosomal translocations or gene amplifications (Figure 4)—these tumors are termed chromosomal unstable (CIN) tumors.²⁷ The multistep model of adenoma progression to carcinoma proposed by Fearon and Vogelstein nearly two decades ago, outlines the contribution of somatic mutation events to the pathogenesis of CRC and continues to serve as a meaningful, although incomplete model of the development of CIN tumors (Figure 4a).²⁸ By contrast, MSI CRC tend to show a stable karyotype (CIN negative), and genetic instability in this subtype of CRC primarily reflects variation in microsatellite tracts owing to a defective MMR system (Figure 4b). More than 30 genes have mutations arising in microsatellite repeats in MMR deficient tumors, and these genes are implicated in diverse cellular functions and pathways. Some examples are the DNA repair proteins MRE11A and hRAD50, the growth factors TGF- β receptor II and IGF receptor II, the pro-apoptotic factor BAX, the mismatch repair proteins MSH3 and MSH6 and the histone modifier HDAC2.²⁹ Although mutations in these pathways are not unique to MSI CRC, the importance of the resultant events could lead to ways of exploiting these specific genes and pathways as potential drug targets and markers of sensitivity to therapies.

Mutations in genes encoding kinases are attractive for clinical researchers because of their notable contribution to tumorigenesis and potential therapeutic value. For example, *BRAF* encodes a serine/threonine kinase that acts upstream of MAPKK1 and MAPKK2 in response to RAS signals, and is an essential component of the RAF/MEK/ERK/MAPK kinase signaling cascade. *BRAF* mutations are present in 10% of CRC and are mutually exclusive with *KRAS* mutations.^{30,31} Most of the mutations in *BRAF* are located in a hotspot located in exon 15, which leads to a V600E single-amino-acid substitution. Strikingly, *BRAF* mutations in colorectal tumors are more frequent in sporadic MSI tumors than in hereditary cases, and are caused by hypermethylation of *MLH1*.^{31,32} Moreover, clinicians have taken advantage of the absence of *BRAF* mutations in Lynch syndrome cases to rule out the sporadic origin of MSI tumors displaying a genetic background.^{33,34} Overall, this fact highlights the strong association that exists between sporadic MSI and the presence of the V600E *BRAF* mutation. Although a *BRAF* mutation profoundly reduces the probability of

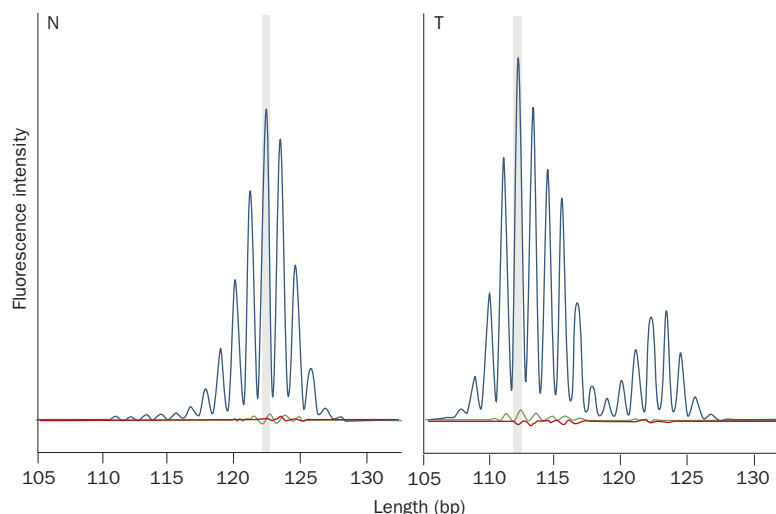


Figure 2 | Capillary electrophoresis of an MSI cancer. Electropherograms of the fluorescent amplification products for the loci BAT25 from normal colon mucosa (N) and CRC tissue (T). This loci contains (A)₂₅ repeats. Electropherograms can identify MSI by the appearance of new shorter peaks due to the shortening of the adenine repeats in cancer cells. The residual normal signal is under-represented compared with the new signal that demonstrates instability, as most of the analyzed tissue is composed of neoplastic cells. Abbreviations: bp, base pairs; CRC, colorectal cancer; MSI, microsatellite instability.

Table 1 | Immunohistochemical patterns of mismatch repair deficiency

Protein				Interpretation	Inactivated gene	Microsatellite instability
<i>MLH1</i>	<i>MSH2</i>	<i>MSH6</i>	<i>PMS2</i>			
+	+	+	+	Intact MMR	None	MSS
+	–	–	+	Deficient MMR	<i>MSH2</i> *	MSI
+	+	–	+	Deficient MMR	<i>MSH6</i>	MSI or MSS
+	+	+	–	Deficient MMR	<i>PMS2</i>	MSI
–	+	+	–	Deficient MMR	<i>MLH1</i> †	MSI

*Lack of MSH2 and MSH6 expression is usually due to a germline mutation in *MSH2*, although it can also be caused by transcriptional read-through of the neighboring *EPCAM* gene, which inactivates *MSH2*. †*MLH1* can be inactivated by germline mutation or hypermethylation of the *MLH1* promoter. Methylation of the *MLH1* promoter is typically accompanied by *BRAF* V600E somatic mutations. Abbreviations: +, present; –, absent; MMR, mismatch repair; MSI, microsatellite instability; MSS, microsatellite stable.

a diagnosis of Lynch syndrome, it does not entirely exclude the possibility.^{35–37} *KRAS* mutations, however, are more likely to be observed in MSS (CIN) cancers than MSI tumors, which is consistent with the original description of these mutations as early key events that lead to intermediate adenomas within the pathogenesis of sporadic CRC.²⁸ Indeed, *KRAS* mutations are present in approximately 40% of colorectal primary tumors.³⁸ Thus, MSI tumors generally have fewer *KRAS* mutations than MSS tumors, and the mutational pattern differs in CIN versus MSI cancers.

In addition to these specific mutational profiles, a description of the genomic landscape of CRC points to the phosphatidylinositol 3-kinase (PI3K) pathway as a driver of tumorigenesis.³⁹ Initially, *PIK3CA* was a focus of attention because it has a key regulatory role in this pathway and was reported to be mutated in 30% of colorectal tumors.⁴⁰ Subsequent studies found lower mutation

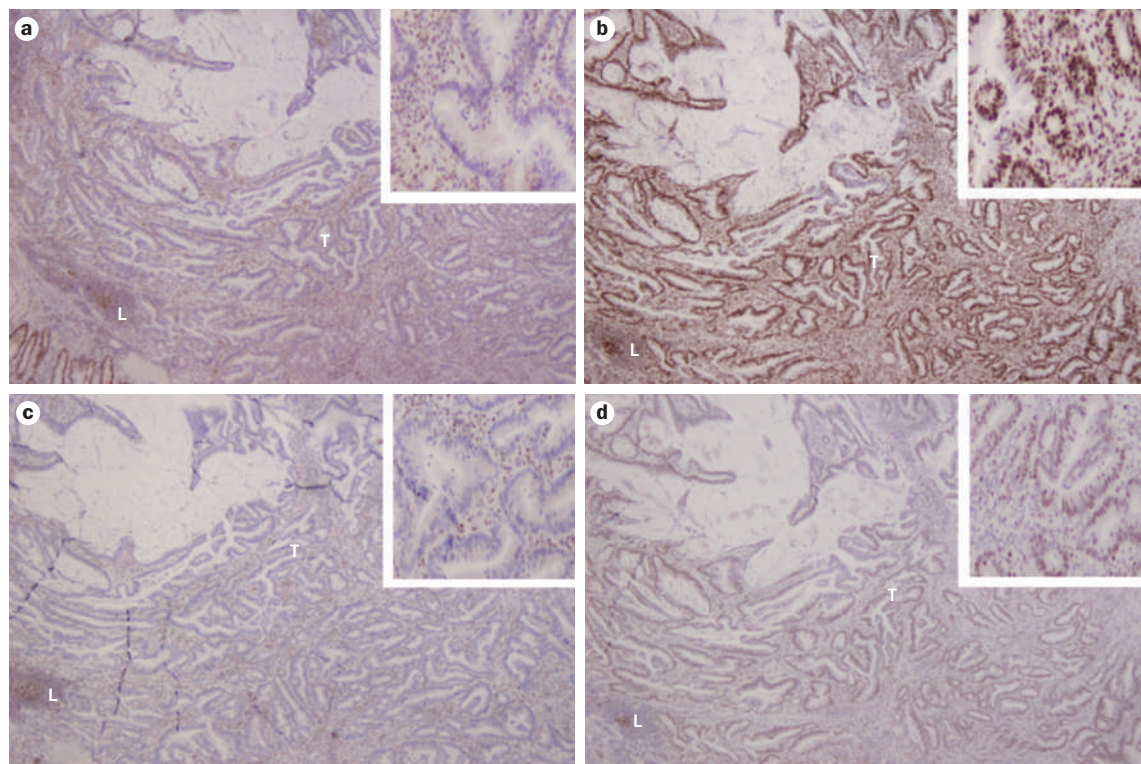


Figure 3 | Immunohistochemical patterns of mismatch repair proteins in colorectal cancers. Absent expression of **a** | MLH1 and **b** | PMS2, shown at magnification $\times 40$ and $\times 100$ in tumor cells, with retained expression in normal colon cells and lymphocytes. Intact expression of **c** | MSH2 and **d** | MSH6 shown at magnification $\times 40$ and $\times 100$ in tumor cells and lymphocytes. Abbreviations: L, lymphocytes; T, tumor.

rates (10–20%) in different populations, although most of these studies only examined mutational hotspots in exons 9 and 20.^{41–47} Furthermore, genes encoding kinases with a less prominent role in this signaling pathway, such as *PDK1*, *AKT2*, *PAK4*, *IRS2*, *INSRR*, *ERBB4* and *PTEN*, are also mutated in CRC, raising the possibility that approximately 40% of colorectal tumors have alterations in one of the PI3K pathway components.⁴⁸ MSI tumors were initially thought to have more mutations in *PIK3CA* than in MSS CRC,^{40,47} but these results have not been confirmed. Discrepancies could be related to differences in tumor stage, patient ethnicity and numbers of exons screened. *PTEN*, which is the only tumor suppressor in this pathway, is not only mutated but also epigenetically silenced with higher frequency in MSI-H tumors than MSI-L or MSS tumors (19.1% versus 2.2%).^{48,49}

Gene-expression profiling of MSI

Several groups have studied the differences in gene expression between MSI and MSS in tumors^{50–54} and cell lines⁵⁵ by use of high-throughput technologies. Most of these studies excluded tumors displaying MSI-L or grouped them with MSS cases.^{50,51,53} In addition, only one of these studies included sufficient Lynch syndrome cases to enable assessment of the differences in gene expression between familial and sporadic MSI cases.⁵⁴ The diversity of the genetic profiles generated from these studies could be related to technical differences in experimental

approaches, selective reporting of fractions of differentially expressed transcripts, or true differences in the expression profiles of the tumors represented in these studies.⁵⁶ Studies evaluating the prognostic performance of gene signatures in breast cancer have driven important clinical trials, such as MINDACT (NCT00433589)⁵⁷ and the TAILORx trial (NCT00310180).⁵⁸ Table 2 summarizes the data generated from six groups describing gene-expression profiles for MSI tumors. The partial overlap of the gene list is notable, especially since many were generated using the same gene platform approach. Moreover, enrichment analyses derived from these profiles have shown that the overlapping features of the expression patterns are linked to immune responses and interleukin pathways. These pathways are related to the high numbers of tumor-infiltrating lymphocytes observed in MSI tumors.⁵⁹ This observation is consistent with the strong immune response that MSI tumors elicit in the host, which can be observed histologically.

In contrast to the small overlap of gene lists assessed in some studies, high levels of reproducibility and concordance between several MSI data sets have also been reported.⁶⁰ Reanalysis of published data sets with new parameters has yielded a common level of significance for all of the data sets ($P < 0.05$). Moreover, this high level of concordance between different MSI data sets was extended to the comparison of tumor samples and established cell line counterparts. These results highlight

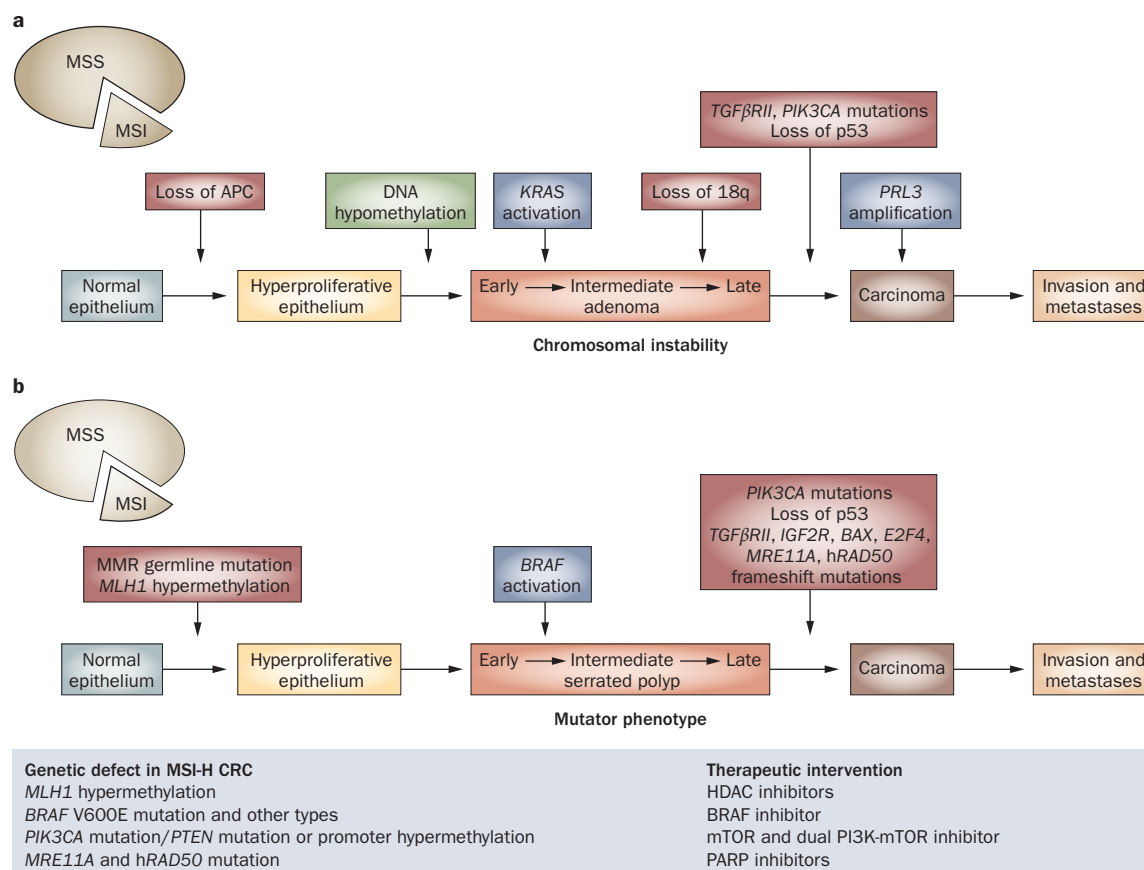


Figure 4 | CRC progression models and therapeutic targets in MSI and MSS CRC. Molecular CRC groups based on **a** | chromosomal instability and **b** | the mutator phenotype. The genetic models for CRC tumorigenesis are presented in parallel for each pathway of tumor development. Targeted therapies based on molecular events are also presented for MSI tumors. Abbreviations: CRC, colorectal cancer; MSI, microsatellite instability; MSS, microsatellite stable.

three principles of gene-expression-profiling studies: direct comparison of expression profiles generated from different sample sets, laboratories and platforms could give a false sense of inconsistency; the limited number of samples of any individual expression profiling study emphasizes the importance of public access to the original expression data; and further statistical method development is warranted to improve meta-analytical approaches for large expression data sets and find the optimal way to combine the analytical results from these different data sets. This last point is especially critical, not only for expression analysis in MSI CRC, but also for other relatively small subtypes, such as Lynch syndrome cases as well as sporadic MSI cancers with or without *BRAF* mutations. Power calculations show that a minimum of 56 tumors per group is needed to achieve a false discovery rate of <5% for a signature of 200 genes with a twofold difference between two groups.⁵⁶ This sample size is large for these subtypes as the incidence is <15% of all CRC. Therefore, meta-analyses combining gene-expression data of samples from different sources and studies would make these comparisons more feasible.

Finally, high-throughput technologies could help to provide more information on two important issues: what forms of gene expression are different between familial

and sporadic MSI tumors, and whether MSI-L is a separate subtype to MSS to define whether MSI follows a trimodal distribution (MSI-H versus MSI-L versus MSS) or a bimodal distribution (MSI versus MSS).²⁵ Until this issue is clarified, combining MSI-L with MSS for outcome studies remains reasonable given that MSI-L cases resemble MSS tumors rather than MSI tumors.

Features and applications of MSI

A recognizable clinicopathological profile of MSI tumors has been established from clinical studies. CRC displaying MSI tend to be right-sided and diagnosed at lower pathological stages compared with MSS cancers. The age distribution of MSI cancers follows a U-shaped distribution, and sporadic MSI cases are generally diagnosed in older patients (>70 years of age) whereas familial cases are younger (<50 years of age).⁶¹ CRC generally have high histological grades, mucinous phenotypes with prominent numbers of tumor-infiltrating lymphocytes, a lack of necrotic cellular debris within the lumen of the neoplastic glands in the colorectal mucosa (dirty necrosis) and a Crohn-like host response. These features can be successfully combined to predict the likelihood of the presence of MSI in tumor samples.^{17,59,62} The MSI phenotype has three major clinical applications: prognosis of

Table 2 | Gene-expression studies characterizing MSI CRC and cell lines

Reference	Expression platform	Number of MSI-H versus MSS	HNPCC	Gene classifier (n)	Molecular pathways
Koinuma <i>et al.</i> (2004) ⁵¹	Affymetrix U133A&B	10/10	No	24	Wnt, Axin-2, Catenin β 1
Banerjee <i>et al.</i> (2004) ⁵⁰	Affymetrix U133A	27/104	Yes	1,293	Immune response, HSP-70, HSP-110, interleukins, apoptosis
Kruhøffer <i>et al.</i> (2005) ⁵⁴	Affymetrix U133A	34/67	Yes	9	–
Watanabe <i>et al.</i> (2006) ⁵³	Affymetrix U133A&B	33/51	No	177	Apoptosis, immune response, mucin
Giacomini <i>et al.</i> (2005) ⁵⁵	Stanford Functional Genomics	8/10	Yes	8	Metallothioneins
Vilar <i>et al.</i> (2009) ⁵²	Affymetrix Hu6800	13/38	Yes	71	–

The GeneChip® human genome arrays were from Affymetrix (Santa Clara, CA, USA).^{51–54} cDNA microarrays were from Stanford Functional Genomics Facility (Stanford, CA, USA).⁵⁵ Abbreviations: Axin-2, axis inhibition protein 2; HNPCC, hereditary nonpolyposis colorectal cancer; HSP, heat shock protein; MSI-H, microsatellite instability-high; MSS, microsatellite stable.

CRC, prediction of response to chemotherapeutic agents, such as 5-fluorouracil (5-FU) and irinotecan, and genetic assessment of Lynch syndrome.

Prognostic value of MSI in CRC

Gryfe *et al.*¹⁷ reported the first cohort of patients in whom the prognostic importance of defective MMR was demonstrated. MSI tumors had a more favorable prognosis and were less prone to lymph-node spread and metastasis than MSS tumors. These results have been corroborated by many subsequent studies, including the large series reported by Watanabe *et al.*⁶³ A meta-analysis assessed individual clinical data from 32 studies that included 7,642 CRC cases, where 1,277 of these cases displayed MSI.⁶⁴ This meta-analysis confirmed the prognostic advantage of MSI. Moreover, a presentation at the ASCO 2009 Annual Meeting reported that the prognostic value of MSI is more prominent in stage II than stage III CRC cases.¹⁹ Despite the reproducibility of these data, MSI testing has not been routinely incorporated into practice to inform patients about their prognosis or to guide therapeutic decision-making.

MSI as a predictor of 5-FU efficacy

The value of MSI as a predictive marker of response to 5-FU, irinotecan and other chemotherapeutic agents remains controversial. Conflicting results have been published during the past decade and are summarized in Table 3.^{62,65–74} The value of MSI as a marker of response to 5-FU was described in a study where MSI cancers were associated with better response to a 5-FU-based regimen than MSS cancers.⁶⁶ This observation is not supported by subsequent studies. Several factors could explain the differences in these results. First, sample sizes are generally too small to show an effect of chemotherapy treatments in patient subgroups stratified by MMR status.⁶⁶ This point

has been addressed in a meta-analysis that confirmed the lack of predictive value of MSI for outcomes with 5-FU regimens.⁷⁴ Second, the results may have been affected by the retrospective and single institutional nature of most of the studies, which used different methods and criteria to evaluate the presence of MSI. Finally, the inadequate interpretation of the data might have been a contributing factor to these results. Interpretation of data is particularly relevant as these studies analyzed the effects of 5-FU in patients with MSI-H CRC. Ideally, predictive data analysis should be performed for every tumor subtype. This method would approximate a clinical trial, where the intervention group is compared with the control group and stratified by MMR status. This approach has been effectively utilized in several, but not all studies of MSI.^{65,69,70,73}

The data presented by Sargent *et al.*⁷³ at the 2008 ASCO Annual Meeting are particularly noteworthy. This large retrospective study included samples obtained from six randomized, controlled trials analyzing the benefit of 5-FU-based adjuvant chemotherapy in patients with stage II and III CRC. Regimens combining oxaliplatin, irinotecan and oral fluoropyrimidines were excluded. In addition, analysis of the efficacy of 5-FU in MSI cases was restricted to stage II patients because administration of adjuvant chemotherapy to stage III patients is currently the standard of care. Sargent *et al.*⁷³ concluded that patients receiving 5-FU had no advantage over those who did not receive 5-FU and this treatment might even be harmful for stage II cases displaying MSI. These findings were, however, interpreted specifically for stage II cancers and generalization to other stages might not be possible. Adjuvant combined chemotherapy with a 5-FU-based regimen remains the standard of care in patients diagnosed with stage III disease, regardless of MSI status. An ongoing clinical trial is exploring the role of MSI and loss of 18q as predictive factors to guide therapeutic decisions on the use of adjuvant therapy for stage II cases (ECOG-E5202).⁷⁵ The study design has been criticized, however, because the results from the PETACC-3 study showed that loss of 18q has no prognostic value for stage II CRC.¹⁹ Definitive interpretation of the value of MSI as a predictive factor for 5-FU-based chemotherapy based on this trial will, therefore, be difficult.

Finally, the predictive value of MSI for 5-FU-based therapies has been debated as there are reports of increased sensitivity to this drug in tumors displaying CpG island methylator phenotype (known as CIMP-High).⁷⁶ Such divergence could be related to the fact that the CIMP-High subtype includes other tumors with molecular features that are different to MSI, such as hypermethylation of *MGMT*.⁷⁷

MSI as a predictor of irinotecan efficacy

Evidence supporting the preferential efficacy of irinotecan in MSI tumors is continuing to emerge, but is still considered preliminary.^{78–81} This chemotherapy agent is a semisynthetic, water-soluble camptothecin analog and a potent inhibitor of the topoisomerase I enzyme. Topoisomerase I generates a transient nick in one of the

Table 3 | MSI as a predictive marker of response to 5-FU-based chemotherapy

Reference	Type of study	Number of patients	Stage	MSI-H (%)	Duration of follow-up (months)	Stratification	5-FU effects	Primary end point	Chemotherapy regimen
Elsaleh <i>et al.</i> (2000) ⁶⁶	R	656	III	8.5	54	MSI-H	Benefit	5 year OS	5-FU + LEV
Hemminki <i>et al.</i> (2000) ⁶⁷	P NR	95	III	12	31	MSI-H	Benefit	3 year DFS	5-FU + FO 5-FU + MTX 5-FU + LEV 5-FU
Liang <i>et al.</i> (2002) ⁶⁸	P NR	244	IV	21.3	–	MSI-H	Benefit	mOS	5-FU + FO high dose
Ribic <i>et al.</i> (2003) ⁶²	R from RCT	570	II–III	16.7	88.8	CT	Detriment	5 year OS/DFS	5-FU + FO 5-FU + LEV
Carethers <i>et al.</i> (1999) ⁶⁵	R	204	II–III	17.6	43.7	CT	No benefit	mOS	5-FU based
Benatti <i>et al.</i> (2005) ⁶⁹	R	1,263	All stages	20.3	64	CT	No benefit	5 year OS	5-FU based
Jover <i>et al.</i> (2006) ⁷⁰	P NR	754	All stages	8.8	24.3	CT	No benefit	mDFS/ mOS	5-FU based
Lamberti <i>et al.</i> (2007) ⁷²	P NR	416	All stages	12.5	32.9	MSI-H	No benefit	mOS	5-FU based
Kim <i>et al.</i> (2007) ⁷¹	R from RCT	542	II–III	18.1	60	CT	No benefit	mDFS/ mOS	5-FU based
Sargent <i>et al.</i> (2008) ⁷³	R from RCT	1,027	II–III	16	60	CT	Detriment	mDFS/ mOS	5-FU + FO
Des Guetz <i>et al.</i> (2009) ⁷⁴	MA	3,690	II–III	14	–	MSI-H	No benefit	DFS/OS	5-FU based

Note that the majority of these trials are observational studies and some of them have retrospectively reviewed tumors collected in the context of randomized, controlled trials. Abbreviations: CT, chemotherapy; DFS, disease-free survival; FO, folinic acid; 5-FU, 5-fluorouracil; LEV, levamisole; MA, meta-analysis; mDFS, median disease-free survival; mOS, median overall survival; MSI-H, microsatellite instability-high; MTX, methotrexate; NR, non-randomized; OS, overall survival; P, prospective; R, retrospective; RCT, randomized controlled trial.

DNA strands during replication and transcription, which allows the DNA to relax. Irinotecan binds to the DNA–topoisomerase I complex and traps it, thus preventing religation of the DNA. The replication and transcription machinery collides with the DNA–topoisomerase I complex, generating a double-strand break. If this dysfunction is unresolved by the DNA repair system, it eventually leads to apoptosis and cell death.⁸² Double-strand breaks can be repaired by two different mechanisms: homologous recombination and nonhomologous end joining. Homologous recombination is activated after replication when a second identical DNA copy is available, making this pathway error-free. Nonhomologous end joining occurs when no DNA template is available and this system is prone to errors. Mechanisms involved in homologous recombination are complex and beyond the scope of this article, but the function of the MRE11A/hRAD50/NBS1 (MRN) complex in this pathway should be highlighted based on its potential role in the response to irinotecan.

Four preclinical studies have suggested that MSI cell models are especially sensitive to irinotecan compared with their proficient counterparts.^{78–81} Although the molecular basis of this increased sensitivity is not fully defined, MSI CRC cell lines tend to accumulate mutations in microsatellites located in an intron–exon boundary poly-T(11) repeat in *MRE11A* and in a coding poly-A(9) tract in *hRAD50*. Cell lines harboring these

mutations also show a particularly high sensitivity to irinotecan.⁷⁸ Moreover, functional work by Rodriguez *et al.*⁸¹ shows that *MRE11A* deficiency or mutations promote sensitivity to camptothecins. The MSS CRC cell line SW-480 was treated with small interfering RNA directed to *MRE11A* or was stably transfected, and these manipulations led to a frameshift mutation in the *MRE11A* gene. Transfected cells were more sensitive to camptothecins than their wild-type counterparts.⁸¹ These experiments provide evidence that secondary mutations in genes that control repair of double-strand breaks are the cause of the increased sensitivity rather than the abnormal MMR itself. An abnormal MMR system does not, however, always result in *MRE11A* deficiency. Mutations in this gene are detected in 70–85% of MSI tumors,^{83,84} making MSI an imperfect surrogate marker for increased sensitivity to irinotecan.

Four clinical studies have analyzed the activity of irinotecan in MSI CRC. The first is a retrospective study analyzing a cohort of 72 patients with metastatic CRC treated with irinotecan in a single institution and stratified by microsatellite status.⁸⁵ The second study is a prospective analysis of a cohort of 702 stage III CRC patients included in the CALGB protocol 89803 study that evaluated the efficacy of a combination of agents (irinotecan, 5-FU and folinic acid) compared with a weekly bolus of 5-FU as adjuvant therapy.⁸⁶ Predictive analysis showed a trend towards a higher benefit of MSI-H tumors treated with

the combined regimen in terms of 5-year disease-free survival, although it did not reach significance. Patients with MSS tumors were not likely to obtain any benefit with the addition of irinotecan to 5-FU.⁸⁶ Data presented by Tejpar *et al.*⁸⁷ at the 2009 ASCO Annual Meeting, however, did not confirm this observation. In this study, a retrospective analysis of 1,254 patients included in the PETACC-3 trial was performed to assess the effect of the folinic acid, 5-FU and irinotecan regimen as adjuvant therapy compared with the de Gramont infusional 5-FU and folinic acid regimen in stage II and III CRC patients. Among 188 MSI-H cases, those treated with irinotecan did not demonstrate improved survival.⁸⁷ The third study is a retrospective review of data from the CAIRO trial, which included sequential irinotecan-containing treatments for stage IV CRC.²⁰ Unfortunately, because only a limited number of the CRC cases were MSI tumors (14 of 515) and both treatment arms included irinotecan at some point, clear interpretation of the MSI data was not possible. Finally, a meta-analysis on the effect of chemotherapy in stage IV patients displaying MSI, including the studies by Fallik *et al.*⁸⁵ and the CAIRO trial,⁸⁸ did not achieve enough power to draw any conclusions about the effect of irinotecan-based regimens.⁸⁹ More studies need to be carried out to clarify the role of chemotherapy regimens containing irinotecan in MSI tumors.

Genetic assessment of Lynch syndrome

The role of MSI as a genetic marker of Lynch syndrome is well established in the clinic. Both MSI detection and immunohistochemistry are highly sensitive methods for the identification of individuals with defective MMR, and guide clinicians towards informative, cost-effective genetic testing. Data are, however, currently insufficient to enable recommendation of a strategy based on one technique or a preferred sequence of both for the detection of MSI tumors in a population.⁹⁰

Tailored therapies for MSI tumors

The development of therapeutic strategies for MSI tumors has traditionally followed a process centered on signaling pathways. The identification of a molecular event could lead to the development of an agent that exploits the deficiency or the hyperactivation of pathways that are caused by specific molecular events; for example, PARP-1 inhibition in *BRCA1* and *BRCA2* mutant breast and ovarian cancers, or *KIT* mutations in gastrointestinal stromal tumors. Gene expression data have been implemented as a way to improve and refine the drug discovery process. The identification of deregulated pathways or gene-expression profiles defining subtypes of tumors has led to the possibility of developing compounds according to the concept of genomic-centered therapeutics.⁹¹

As an example, we have found evidence of pathway-centered signaling in MSI CRC.⁹² PARP-1 inhibitors represent an attractive target in MSI tumors since there seems to be a deficient double-strand-break repair system related to mutations in the coding microsatellites of *hRAD50* and *MRE11A*. The effect of PARP-1 inhibitors in MSI cell lines were tested, therefore, and the

expression level of *MRE11A* was correlated with drug response.⁹² Although these data need to be confirmed in other settings, they suggest that specific mutations can be used to exploit the concept of synthetic lethality in MSI tumors, which has been successful in *BRCA1*-mutant breast cancers.⁹³ Another example explores the activity of demethylating agents in MSI cell line models. A particular frameshift mutation of *HDAC2* located in a coding poly-A9 repeat of exon 1 determined the lack of response in MSI cells to certain histone deacetylase inhibitors, such as trichostatin A, but not to others, such as butyric acid and valproic acid.⁹⁴ This result is due to biochemical differences that limit the access of these drugs to catalytic sites. These two examples demonstrate that genetic instability owing to MSI could represent an Achilles' heel of specific subtypes of cancer that can be therapeutically targeted.

Despite incomplete overlap, gene-expression data might have the potential to identify new therapies for MSI tumors.⁵² With the use of a systems biology tool called Connectivity Map, it was shown that both sirolimus and LY-294002 (compounds that target the PI3K/AKT/mTOR pathway) induce gene expression changes with negative correlation to expression patterns in MSI tumors. Sirolimus and LY-294002 were then confirmed *in vitro* to be selective inhibitors for MSI cell lines. These results highlight the relevance of the PI3K/AKT/mTOR pathway in this tumor subtype and have uncovered new potential targets for drug discovery.⁵²

Although CRC comprise a heterogeneous group of cancers, recognition of the MSI phenotypes draws attention to the value of understanding specific subtypes of cancer. The data suggest it is worthwhile devoting substantial efforts towards designing clinical trials for CRC subtypes and assessing the efficacy of compounds that have mechanisms of action linked to specific molecular characteristics based on data generated not only from single-gene, but also pathway and genome-centered discoveries. An ongoing phase II clinical trial (CINATRA) is exploring the effect of a new microtubule inhibitor patupilone (EP0906) in a cohort of patients displaying MSI.⁹⁰ This trial is based on the hypothesis that karyotypically stable colorectal tumors (CIN positive) will be more sensitive to antimicrotubule agents than karyotypically unstable tumors (CIN negative). This study and others like it represent an important paradigm for advancing treatment options for patients with CRC.

Conclusions

MSI is a molecular subtype of CRC that displays a well-defined histopathological and therapeutic profile distinct from other molecular subtypes. Molecular techniques developed during the past two decades allow reliable detection of MSI. Although these techniques are accessible to molecular pathology laboratories, the oncology community has yet to fully embrace MSI detection in routine clinical practice with respect to its prognostic and predictive role. Emerging data from well-designed clinical trials should provide further support for clinical utility and implementation.

Drug development strategies focused on specific molecular subtypes clearly represent the future of cancer therapeutics. Clinical trials based on molecular classifications are likely to have stronger rationales than prior studies, and will increase the likelihood of obtaining a relevant therapeutic response, thus improving the health and well-being of patients. MSI CRC are ideal for the implementation of this strategy based on a compelling rationale motivated by molecular, clinical, pathological, prognostic and predictive studies that are already changing the practice of oncology.

Review criteria

The articles for this Review were identified by searching the PubMed database for relevant publications written in English and published before 1 May 2009. The search terms were "microsatellite instability", "colorectal cancer", "PI3K", "BRAF", "gene expression profile", "prognosis", "5-fluorouracil" and "irinotecan". In addition, proceedings from ASCO conferences published before 20 May 2009 were searched for relevant abstracts. References were chosen based on the best clinical evidence that addressed this article's objectives.

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