Published in final edited form as:
Nat Rev Clin Oncol. 2010 March ; 7(3): 153–162. doi:10.1038/nrclinonc.2009.237.

Microsatellite instability in colorectal cancer—the stable evidence

Eduardo Vilar and **Stephen B. Gruber**
Department of Internal Medicine, University of Michigan Comprehensive Cancer Center, University of Michigan Medical School, 1524 BSRB, 109 Zina Pitcher, Ann Arbor, MI 48109, USA

Abstract

Microsatellite instability (MSI) is the molecular fingerprint of a deficient mismatch repair system. Approximately 15% of colorectal cancers (CRC) display MSI owing to either 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 with use of irinotecan-based regimens, but these findings require further clarification and are not yet in routine use. Characterization of the molecular basis of MSI CRC is underway and initial results show that mutations in genes controlling 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.

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 applying various approaches have characterized this molecular subtype. Tumors harboring a deficient mismatch repair (MMR) system owing to germline, somatic or epigenetic inactivation account for 15–20% of CRC in the USA.[4,5] Although this proportion represents a large population burden of a specific molecular phenotype, MSI has only slowly been recognized as a clinically relevant aspect of tumor biology whereas it is an established molecular marker for patients with suspected Lynch syndrome. 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 this tumor subtype are discussed.

Molecular basis of the MMR system

Microsatellites are repetitive sequences distributed throughout the human genome and consist of mononucleotide, dinucleotide or higher-order nucleotide repeats such as $(A)_n$ or

Correspondence to: S. B. Gruber, sgruber@umich.edu.

Competing interests
The authors declare no competing interests.

$(CA)_n$. These sequence motifs are especially prone to accumulation of mutations, mainly due to slippage of polymerases 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 partnerless 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 leading to protein truncations.[6]

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, MSH6 and PMS2 are the main proteins involved in this system, and they interact as heterodimers. When a mismatch is detected, three steps take place: MSH2 associates with either MSH6 or MSH3 (forming MutSα and MutSβ complexes, respectively) and MLH1 couples with PMS2, PMS1 or MLH3 (forming MutLα, MutLβ or MutLγ complexes, respectively). The recognition of mismatches and insertion–deletion loops is carried out by a sliding clamp formed by the combination of a MutS and a MutL complex, which interacts with replication factor C. Excision of hExo1 is performed by proteins such as exonuclease-1 and proliferating cell nuclear antigen. Resynthesis and religation is carried out by DNA polymerase δ and DNA ligase.[6,7] 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 colorectal, gastric, endometrial, ovarian and sebaceous carcinomas, as well as glioblastoma and lymphomas.[8,9] First discovered in CRC, the MSI phenotype continues to serve as the hallmark of defective MMR since the observation 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).[10,11] In addition, a heritable somatic methylation of *MSH2* has been reported, which is caused by a deletion of the last exon of *EPCAM*, which is adjacent to *MSH2*.[12] Lynch syndrome is an autosomal dominant disease that accounts for 2–3% of total CRC.[4,5,13] If germline mutations are harbored in MMR genes, 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] However, most cases of MSI CRC are owing to 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 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 are 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 (markers). The presence of instability is determined by comparison of the length of nucleotide repeats in tumor cells and normal cells. Normal DNA is typically extracted from adjacent normal mucosa. This technique was initially performed with denaturing acrylamide gels and radiolabeled primers; however, this process has been made easier with fluoresceinated 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.[8,26] 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 CRC as MSI-H. On the contrary, mononucleotides BAT25 and BAT26 are nearly monomorphic, so in the absence of normal control tissue, MSI determination could be based entirely on these.[25] Therefore, the Bethesda panel has a sufficient combination of markers for MSI detection, but newer commercial kits now 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 proteins 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 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.[11] As an example, CRCs 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 concentrate on *MLH1* rather than the other MMR genes.

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 relative deficiencies 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), and are, therefore, termed chromosomal instable (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 CRCs 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 deficient MMR 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 TGFB receptor II and IGF receptor II, the pro-apoptotic factor BAX, the mismatch repair genes *MSH3* and *MSH6* and the histone modifier HD2.[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/or markers of sensitivity to therapies.

Kinase mutations are attractive for clinical researchers because of their notable contribution to tumorigenesis and potential therapeutic value. *BRAF,* for example, 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 cascade. *BRAF* mutations are present in 10% of CRC and are mutually exclusive with *KRAS* mutations.[30,31] Most of these mutations are located in a hotspot located in exon 15 that leads to a V600E single-amino-acid substitution. Strikingly, *BRAF* mutations in colorectal tumors are more frequent in sporadic MSI tumors caused by hypermethylation of *MLH1*[31,32] than in the hereditary cases. 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 *BRAF* mutation expressed in V600E. Although positive results on *BRAF* testing guides clinicians away from a diagnosis of Lynch syndrome, it does not entirely exclude the possibility.[35–37] *KRAS* mutations, on the other hand, 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 leading 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 PI3K pathway as a 'driver' of tumorigenesis.[39] Initially, *PIK3CA* was the 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 rates (10–20%) in different populations, although most of these studies only examined mutational hotspots in exons 9 and 20.[41–47] Furthermore, other genes controlling kinases with a less prominent role in this signaling pathway (such as *PDK1*, *AKT2*, *PAK4*, *IRS2*, *INSRR* and *ERBB4)* and the *PTEN* gene are also mutated in CRC, raising the point that approximately 40% of colorectal tumors have alterations in one of the PI3K pathway genes.[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 stages, 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 high frequency in MSI tumors.[48,49]

Gene expression profiling of MSI tumors

Several groups have studied the differences in gene expression between MSI and MSS in tumors[50–54] and cell lines[55] 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] This topic has been highlighted because trials evaluating the prognostic performance of gene signatures in breast cancer now drive important clinical trials, such as MINDACT (NCT00433589) and the TayloRx trial (NCT00310180).[57] Table 3 summarizes the data generated from five groups describing gene profiles for MSI tumors. The partial overlap of the gene list is notable, especially since many were generated on the same platform. 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 two pathways are related to the high numbers of tumor-infiltrating lymphocytes observed in MSI tumors.[58] 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 papers, high levels of reproducibility and concordance between several MSI data sets have also been reported.[59] Reanalysis of published datasets with new normalizations of the original expression data has yielded a common level of significance for all of the data sets (P=<0.05). Moreover, this high level of concordance was extended to the comparison of tumor samples and established cell-line counterparts. These results highlight three principles of gene expression profiling studies: direct comparison of expression results generated from different samples sets, laboratories and platforms could give a false sense of inconsistency; the limited numbers 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-analytic approaches for large expression data sets and the optimal way to combine them. 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, for which 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 and, therefore, whether MSI follows a trimodal distribution (MSI-H versus MSI-L versus MSS) or a bimodal distribution (MSI versus MSS).[25] Until this latter point is clarified, the combining of 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. Regarding the age at diagnosis, sporadic MSI cases are generally diagnosed in older patients (> 70 years of age), and familial cases are younger (< 50 years of age) and show a U-shaped age distribution.[60] Under the microscope CRC generally have high histological grades, mucinous phenotypes with prominent numbers of tumor-infiltrating lymphocytes, a lack of 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.[17,58,61]

The MSI phenotype has three major clinical applications. Prognosis of CRC, prediction of response to chemotherapeutic agents, such as 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 and distant metastatic spread than MSS tumors. These results have been corroborated by many subsequent studies, such as that in the large series reported by Watanabe *et al.*[62] Individual clinical data from a total of 32 studies were considered in a

meta-analysis that included 7,642 cases, where 1,277 of these displayed MSI. The meta-analysis confirmed the prognostic advantage of MSI.[63] 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 cases.[19] Despite the reproducibility of these data, they have not been routinely incorporated into practice for MSI to inform patients about their prognosis or to guide therapeutic decision-making.

MSI as a predictor of therapeutic 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 2.[61,65–74] Elsaleh *et al.*[66] described the value of MSI as a marker of response to 5-FU. In this study, MSI was associated with better response than MSS cancers to a 5-FU-based regimen, but this observation seems not to be supported by subsequent studies. Several factors might explain the divergence in results, such as in the Elsaleh *et al.* study the sample size was too small to show an effect of chemotherapy treatments in patient subgroups stratified by MMR status. 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] the retrospective and single institutional nature of most of the studies included different methods and criteria to evaluate the presence of MSI across the studies; the inadequate interpretation of the data might all have been contributing factors to this result. 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, stratified by MMR status. This approach has been effectively utilized in several studies, but not all studies of MSI.[65,69,70,73]

Attention should be brought to the results presented by Sargent *et al.* at the 2008 ASCO Annual Meeting.[73] This large retrospective study includes samples obtained from six randomized, controlled trials analyzing the benefit of 5-FU-based adjuvant chemotherapy in 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 were restricted to stage II patients as administration of adjuvant chemotherapy to stage III patients is currently the standard of care. Sargent *et al.* concluded that 5-FU has no advantage over no 5-FU and might even be harmful for stage II cases displaying MSI,[73] however, these findings were 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 as having stage III disease, regardless of MSI status. An ongoing clinical trial is exploring the roles of MSI and 18q loss as predictive factors to guide therapeutic decisions on the use of adjuvant therapy for stage II cases (ECOG-E5202).[75] The study design has, however, been challenged, given that the results from the PETACC-3 study show that loss of 18q has no prognostic value for stage II CRC.[19] Therefore, definitive interpretation of the value of MSI as a predictive factor for 5-FU-based chemotherapy based on this trial will be difficult.

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

Evidence on the effects of irinotecan in MSI tumors is continuing to emerge, but is still considered preliminary.[52, 79–81] Irinotecan is a semisynthetic, water-soluble camptothecin analog, originally isolated from bark of the tree *Camptotheca acuminata*. This chemotherapy

agent is a potent inhibitor of the topoisomerase I enzyme. Topoisomerase I generates a transient nick in one of the 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.[78] Double-strand breaks are repaired through two different models: 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 Review, but the function of the MRN complex (MRE11A, hRAD50 and NBS1) in this pathway should be highlighted owing to 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.[52,79–81] Although the molecular basis of this increased sensitivity remains partially elusive, 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.[52] Moreover, elegant 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, leading to a frameshift mutation in the *MRE11A* gene. Transfected cells were more sensitive to camptothecins than their wild-type counterparts. These experiments provide evidence that secondary mutations 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,[82,83] 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.[84] The second study is a prospective analysis of a cohort of 702 stage III patients included in the CALGB protocol 89803, evaluating the efficacy of a combination of agents (irinotecan, 5-FU and folinic acid) compared with a weekly bolus of 5-FU as adjuvant therapy. 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.[85] Data presented by Tejpar *et al.* at the 2009 ASCO Annual Meeting, however, did not confirm this observation. Tejpar *et al.* [86] performed a retrospective analysis of 1,254 patients included in the PETACC3 trial to study 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 patients. Among 188 MSI-H cases, those treated with irinotecan did not demonstrate improved survival. The third study is a retrospective review of the data from the CAIRO trial, which includes sequential treatments containing irinotecan 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 no possible. Finally, a meta-analysis on the effect of chemotherapy in stage IV patients displaying MSI, including the studies by Fallik *et al.* and the CAIRO trial, did not achieve enough power to draw any conclusions about the effect of irinotecan-based

regimens.[87] Therefore, 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 and is now the standard of care. Both MSI detection and immunohistochemistry are highly sensitive methods for the identification of individuals with a defective MMR system, 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.[64]

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 would exploit the deficiency or the hyperactivation of pathways owing to 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.[88]

As an example, we have found evidence of pathway-centered signaling in MSI CRCs.[89] PARP-1 inhibitors represent an attractive target in MSI tumors since there seems to be a deficient double-strand-break repair system owing to mutations in coding microsatellites in *hRAD50* and *MRE11A*. The effect of PARP-1 inhibitors in MSI cell lines were, therefore, tested and the expression level of MRE11A was correlated with drug response.[89] 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.[90] 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.[91] 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.[92] With use of a systems biology tool called Connectivity Map, sirolimus and LY-294002—compounds that both target the PI3K-AKT-mTOR pathway—were shown to 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 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.[92]

Although CRC comprise a heterogenous 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, based on the hypothesis that karyotypically stable colorectal tumors (CIN positive) will be more sensitive to antimicrotubule agents than karyotypically instable tumors (CIN negative).[88] 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 that is 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 daily clinical routine 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 previously performed classical 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 owing to a compelling rationale motivated by molecular, clinical, pathological, prognostic and predictive studies that are already changing the practice of oncology.

Acknowledgments

E. Vilar was supported by a fellowship from “la Caixa”, Barcelona, Spain. S. B. Gruber’s laboratory work is supported by NCI grant 1R01CA81488 and by the University of Michigan Comprehensive Cancer Center Core Support grant NIH 5P30CA46592.

References

1. Aaltonen LA, et al. Clues to the pathogenesis of familial colorectal cancer. Science. 1993; 260:812–816. [PubMed: 8484121]
2. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature. 1993; 363:558–561. [PubMed: 8505985]
3. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. Science. 1993; 260:816–819. [PubMed: 8484122]
4. Aaltonen LA, et al. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. N Engl J Med. 1998; 338:1481–1487. [PubMed: 9593786]
5. Hampel H, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med. 2005; 352:1851–1860. [PubMed: 15872200]
6. Jiricny J. The multifaceted mismatch-repair system. Nat Rev Mol Cell Biol. 2006; 7:335–346. [PubMed: 16612326]
7. Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. Nature. 2001; 411:366–374. [PubMed: 11357144]
8. Boland CR, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res. 1998; 58:5248–5257. [PubMed: 9823339]

9. Pal T, Permuth-Wey J, Kumar A, Sellers TA. Systematic review and meta-analysis of ovarian cancers: estimation of microsatellite-high frequency and characterization of mismatch repair deficient tumor histology. Clin Cancer Res. 2008; 14:6847–6854. [PubMed: 18980979]
10. Vogelstein, B.; Kinzler, KW. The genetic basis of human cancer. McGraw-Hill Medical; New York: 2002.
11. Hendriks YM, et al. Diagnostic approach and management of Lynch syndrome (hereditary nonpolyposis colorectal carcinoma): a guide for clinicians. CA Cancer J Clin. 2006; 56:213–225. [PubMed: 16870997]
12. Ligtenberg MJ, et al. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3′ exons of TACSTD1. Nat Genet. 2009; 41:112–117. [PubMed: 19098912]
13. Salovaara R, et al. Population-based molecular detection of hereditary nonpolyposis colorectal cancer. J Clin Oncol. 2000; 18:2193–2200. [PubMed: 10829038]
14. Chen S, et al. Prediction of germline mutations and cancer risk in the Lynch syndrome. JAMA. 2006; 296:1479–1487. [PubMed: 17003396]
15. Stoffel E, et al. Calculation of risk of colorectal and endometrial cancer among patients with Lynch syndrome. Gastroenterology. 2009; 137:1621–1627. [PubMed: 19622357]
16. Herman JG, et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Natl Acad Sci USA. 1998; 95:6870–6875. [PubMed: 9618505]
17. Gryfe R, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. N Engl J Med. 2000; 342:69–77. [PubMed: 10631274]
18. Piñol V, et al. Accuracy of revised Bethesda guidelines, microsatellite instability, and immunohistochemistry for the identification of patients with hereditary nonpolyposis colorectal cancer. JAMA. 2005; 293:1986–1994. [PubMed: 15855432]
19. Roth AD, et al. Stage-specific prognostic value of molecular markers in colon cancer: Results of the translational study on the PETACC 3-EORTC 40993-SAKK 60-00 trial. ASCO Meeting Abstracts. 2009; 27:4002.
20. Koopman M, et al. Deficient mismatch repair system in patients with sporadic advanced colorectal cancer. Br J Cancer. 2009; 100:266–273. [PubMed: 19165197]
21. Ashktorab H, et al. High incidence of microsatellite instability in colorectal cancer from African Americans. Clin Cancer Res. 2003; 9:1112–1117. [PubMed: 12631615]
22. Kumar K, et al. Distinct BRAF (V600E) and KRAS mutations in high microsatellite instability sporadic colorectal cancer in African Americans. Clin Cancer Res. 2009; 15:1155–1161. [PubMed: 19190129]
23. Soliman AS, et al. Contrasting molecular pathology of colorectal carcinoma in Egyptian and Western patients. Br J Cancer. 2001; 85:1037–1046. [PubMed: 11592777]
24. Gruber SB. New developments in Lynch syndrome (hereditary nonpolyposis colorectal cancer) and mismatch repair gene testing. Gastroenterology. 2006; 130:577–587. [PubMed: 16472609]
25. Laghi L, Bianchi P, Malesci A. Differences and evolution of the methods for the assessment of microsatellite instability. Oncogene. 2008; 27:6313–6321. [PubMed: 18679418]
26. Umar A, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004; 96:261–268. [PubMed: 14970275]
27. Vogelstein B, et al. Allelotype of colorectal carcinomas. Science. 1989; 244:207–211. [PubMed: 2565047]
28. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990; 61:759–767. [PubMed: 2188735]
29. Duval A, Hamelin R. Mutations at coding repeat sequences in mismatch repair-deficient human cancers: toward a new concept of target genes for instability. Cancer Res. 2002; 62:2447–2454. [PubMed: 11980631]
30. Davies H, et al. Mutations of the BRAF gene in human cancer. Nature. 2002; 417:949–954. [PubMed: 12068308]

31. Rajagopalan H, et al. Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. Nature. 2002; 418:934. [PubMed: 12198537]
32. Oliveira C, et al. BRAF mutations characterize colon but not gastric cancer with mismatch repair deficiency. Oncogene. 2003; 22:9192–9196. [PubMed: 14668801]
33. Domingo E, et al. BRAF screening as a low-cost effective strategy for simplifying HNPCC genetic testing. J Med Genet. 2004; 41:664–668. [PubMed: 15342696]
34. Bessa X, et al. A prospective, multicenter, population-based study of BRAF mutational analysis for Lynch syndrome screening. Clin Gastroenterol Hepatol. 2008; 6:206–214. [PubMed: 18096441]
35. Deng G, et al. BRAF mutation is frequently present in sporadic colorectal cancer with methylated hMLH1, but not in hereditary nonpolyposis colorectal cancer. Clin Cancer Res. 2004; 10:191–195. [PubMed: 14734469]
36. McGivern A, et al. Promoter hypermethylation frequency and BRAF mutations distinguish hereditary non-polyposis colon cancer from sporadic MSI-H colon cancer. Fam Cancer. 2004; 3:101–107. [PubMed: 15340260]
37. Wang L, et al. BRAF mutations in colon cancer are not likely attributable to defective DNA mismatch repair. Cancer Res. 2003; 63:5209–5212. [PubMed: 14500346]
38. Andreyev HJ, Norman AR, Cunningham D, Oates JR, Clarke PA. Kirsten ras mutations in patients with colorectal cancer: the multicenter “RASCAL” study. J Natl Cancer Inst. 1998; 90:675–684. [PubMed: 9586664]
39. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer. 2002; 2:489–501. [PubMed: 12094235]
40. Samuels Y, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science. 2004; 304:554. [PubMed: 15016963]
41. Barault L, et al. Mutations in the RAS-MAPK, PI(3)K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. Int J Cancer. 2008; 122:2255–2259. [PubMed: 18224685]
42. Benvenuti S, et al. PIK3CA cancer mutations display gender and tissue specificity patterns. Hum Mutat. 2008; 29:284–288. [PubMed: 18022911]
43. Frattini M, et al. Phosphatase protein homologue to tensin expression and phosphatidylinositol-3 phosphate kinase mutations in colorectal cancer. Cancer Res. 2005; 65:11227. [PubMed: 16322273]
44. Kato S, et al. PIK3CA mutation is predictive of poor survival in patients with colorectal cancer. Int J Cancer. 2007; 121:1771–1778. [PubMed: 17590872]
45. Sartore-Bianchi A, et al. PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. Cancer Res. 2009; 69:1851–1857. [PubMed: 19223544]
46. Ogino S, et al. PIK3CA mutation is associated with poor prognosis among patients with curatively resected colon cancer. J Clin Oncol. 2009; 27:1477–1484. [PubMed: 19237633]
47. Abubaker J, et al. Clinicopathological analysis of colorectal cancers with PIK3CA mutations in Middle Eastern population. Oncogene. 2008; 27:3539–3545. [PubMed: 18193083]
48. Parsons DW, et al. Colorectal cancer: mutations in a signalling pathway. Nature. 2005; 436:792. [PubMed: 16094359]
49. Goel A, et al. Frequent inactivation of PTEN by promoter hypermethylation in microsatellite instability-high sporadic colorectal cancers. Cancer Res. 2004; 64:3014–3021. [PubMed: 15126336]
50. Banerjea A, et al. Colorectal cancers with microsatellite instability display mRNA expression signatures characteristic of increased immunogenicity. Mol Cancer. 2004; 3:21. [PubMed: 15298707]
51. Koinuma K, et al. Mutations of BRAF are associated with extensive hMLH1 promoter methylation in sporadic colorectal carcinomas. Int J Cancer. 2004; 108:237–242. [PubMed: 14639609]
52. Vilar E, et al. Microsatellite instability due to hMLH1 deficiency is associated with increased cytotoxicity to irinotecan in human colorectal cancer cell lines. Br J Cancer. 2008; 99:1607–1612. [PubMed: 18941461]

53. Watanabe T, et al. Distal colorectal cancers with microsatellite instability (MSI) display distinct gene expression profiles that are different from proximal MSI cancers. Cancer Res. 2006; 66:9804–9808. [PubMed: 17047040]
54. Kruhøffer M, et al. Gene expression signatures for colorectal cancer microsatellite status and HNPCC. Br J Cancer. 2005; 92:2240–2248. [PubMed: 15956967]
55. Giacomini CP, et al. A gene expression signature of genetic instability in colon cancer. Cancer Res. 2005; 65:9200–9205. [PubMed: 16230380]
56. Michiels S, Koscielny S, Hill C. Interpretation of microarray data in cancer. Br J Cancer. 2007; 96:1155–1158. [PubMed: 17342085]
57. Cardoso F, et al. Clinical application of the 70-gene profile: the MINDACT trial. J Clin Oncol. 2008; 26:729–735. [PubMed: 18258980]
58. Greenson JK, et al. Phenotype of microsatellite unstable colorectal carcinomas: Well-differentiated and focally mucinous tumors and the absence of dirty necrosis correlate with microsatellite instability. Am J Surg Pathol. 2003; 27:563–570. [PubMed: 12717242]
59. Jorissen RN, et al. DNA copy-number alterations underlie gene expression differences between microsatellite stable and unstable colorectal cancers. Clin Cancer Res. 2008; 14:8061–8069. [PubMed: 19088021]
60. Poynter JN, et al. Associations between smoking, alcohol consumption, and colorectal cancer, overall and by tumor microsatellite instability status. Cancer Epidemiol Biomarkers Prev. 2009; 18:2745–2750. [PubMed: 19755657]
61. Ribic CM, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med. 2003; 349:247–257. [PubMed: 12867608]
62. Watanabe T, et al. Molecular predictors of survival after adjuvant chemotherapy for colon cancer. N Engl J Med. 2001; 344:1196–1206. [PubMed: 11309634]
63. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol. 2005; 23:609–618. [PubMed: 15659508]
64. Engstrom PF, et al. NCCN Clinical Practice Guideline in Oncology: colon cancer. J Natl Compr Canc Netw. 2009; 7:778–831. [PubMed: 19755046]
65. Carethers JM, et al. Mismatch repair proficiency and in vitro response to 5-fluorouracil. Gastroenterology. 1999; 117:123–131. [PubMed: 10381918]
66. Elsaleh H, et al. Association of tumour site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. Lancet. 2000; 355:1745–1750. [PubMed: 10832824]
67. Hemminki A, Mecklin JP, Järvinen H, Aaltonen LA, Joensuu H. Microsatellite instability is a favorable prognostic indicator in patients with colorectal cancer receiving chemotherapy. Gastroenterology. 2000; 119:921–928. [PubMed: 11040179]
68. Liang JT, et al. High-frequency microsatellite instability predicts better chemosensitivity to high-dose 5-fluorouracil plus leucovorin chemotherapy for stage IV sporadic colorectal cancer after palliative bowel resection. Int J Cancer. 2002; 101:519–525. [PubMed: 12237891]
69. Benatti P, et al. Microsatellite instability and colorectal cancer prognosis. Clin Cancer Res. 2005; 11:8332–8340. [PubMed: 16322293]
70. Jover R, et al. Mismatch repair status in the prediction of benefit from adjuvant fluorouracil chemotherapy in colorectal cancer. Gut. 2006; 55:848–855. [PubMed: 16299036]
71. Kim GP, et al. Prognostic and predictive roles of high-degree microsatellite instability in colon cancer: a National Cancer Institute-National Surgical Adjuvant Breast and Bowel Project Collaborative Study. J Clin Oncol. 2007; 25:767–772. [PubMed: 17228023]
72. Lamberti C, et al. Microsatellite instability did not predict individual survival of unselected patients with colorectal cancer. Int J Colorectal Dis. 2007; 22:145–152. [PubMed: 16724208]
73. Sargent DJ, et al. Confirmation of deficient mismatch repair (dMMR) as a predictive marker for lack of benefit from 5-FU based chemotherapy in stage II and III colon cancer (CC): A pooled molecular reanalysis of randomized chemotherapy trials [abstract]. J Clin Oncol. 2008; 26:4008.
74. Des Guetz G, et al. Does microsatellite instability predict the efficacy of adjuvant chemotherapy in colorectal cancer? A systematic review with meta-analysis. Eur J Cancer. 2009; 45:1890–1896. [PubMed: 19427194]

75. Baddi L, Benson A. III. Adjuvant therapy in stage II colon cancer: current approaches. Oncologist. 2005; 10:325–331. [PubMed: 15851790]
76. Van Rijnsoever M, Elsaleh H, Joseph D, McCaul K, Iacopetta B. CpG island methylator phenotype is an independent predictor of survival benefit from 5-fluorouracil in stage III colorectal cancer. Clin Cancer Res. 2003; 9:2898–2903. [PubMed: 12912934]
77. Nagasaka T, et al. Hypermethylation of O6-methylguanine-DNA methyltransferase promoter may predict nonrecurrence after chemotherapy in colorectal cancer cases. Clin Cancer Res. 2003; 9:5306–5312. [PubMed: 14614014]
78. Pommier Y. Topoisomerase I inhibitors: camptothecins and beyond. Nat Rev Cancer. 2006; 6:789–802. [PubMed: 16990856]
79. Magrini R, et al. Cellular effects of CPT-11 on colon carcinoma cells: dependence on p53 and hMLH1 status. Int J Cancer. 2002; 101:23–31. [PubMed: 12209584]
80. Jacob S, Aguado M, Fallik D, Praz F. The role of the DNA mismatch repair system in the cytotoxicity of the topoisomerase inhibitors camptothecin and etoposide to human colorectal cancer cells. Cancer Res. 2001; 61:6555–6562. [PubMed: 11522654]
81. Rodriguez R, et al. Thymidine selectively enhances growth suppressive effects of camptothecin/irinotecan in MSI+ cells and tumors containing a mutation of MRE11. Clin Cancer Res. 2008; 14:5476–5483. [PubMed: 18765539]
82. Giannini G, et al. Mutations of an intronic repeat induce impaired MRE11 expression in primary human cancer with microsatellite instability. Oncogene. 2004; 23:2640–2647. [PubMed: 15048091]
83. Miquel C, et al. Frequent alteration of DNA damage signalling and repair pathways in human colorectal cancers with microsatellite instability. Oncogene. 2007; 26:5919–5926. [PubMed: 17384679]
84. Fallik D, et al. Microsatellite instability is a predictive factor of the tumor response to irinotecan in patients with advanced colorectal cancer. Cancer Res. 2003; 63:5738–5744. [PubMed: 14522894]
85. Bertagnolli MM, et al. Microsatellite instability predicts improved response to adjuvant therapy with irinotecan, fluorouracil, and leucovorin in stage III colon cancer: Cancer and Leukemia Group B Protocol 89803. J Clin Oncol. 2009; 27:1814–1821. [PubMed: 19273709]
86. Tejpar S, et al. Microsatellite instability (MSI) in stage II and III colon cancer treated with 5FU-LV or 5FU-LV and irinotecan (PETACC 3-EORTC 40993-SAKK 60/00 trial) [abstract]. J Clin Oncol. 2009; 27:4001.
87. Des Guetz G, et al. Microsatellite instability does not predict the efficacy of chemotherapy in metastatic colorectal cancer. A systematic review and meta-analysis. Anticancer Res. 2009; 29:1615–1620. [PubMed: 19443375]
88. Swanton C, Caldas C. Molecular classification of solid tumours: towards pathway-driven therapeutics. Br J Cancer. 2009; 100:1517–1522. [PubMed: 19367275]
89. Vilar E, et al. Preclinical testing of the PARP inhibitor ABT-888 in microsatellite instable colorectal cancer [abstract]. J Clin Oncol. 2009; 27:11028.
90. Farmer H, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. 2005; 434:917–921. [PubMed: 15829967]
91. Ropero S, et al. A truncating mutation of HDAC2 in human cancers confers resistance to histone deacetylase inhibition. Nat Genet. 2006; 38:566–569. [PubMed: 16642021]
92. Vilar E, et al. Gene expression patterns in mismatch repair-deficient colorectal cancers highlight the potential therapeutic role of inhibitors of the phosphatidylinositol 3-kinase-AKT-mammalian target of rapamycin pathway. Clin Cancer Res. 2009; 15:2829–2839. [PubMed: 19351759]

Biographies

Eduardo Vilar is a Postdoctoral Research Fellow at the University of Michigan, Ann Arbor, USA. He received his MD and PhD from Miguel Hernandez University Medical School, Alicante and completed his medical oncology training at Vall d'Hebron University Hospital, Barcelona, both in Spain. He received a masters degree in molecular oncology from the Spanish National Cancer Research Center, Madrid, Spain. He was awarded a Merit Award

from the American Society of Clinical Oncology Foundation in 2008 and 2009. His research interests include the molecular biology of colorectal tumors and the application of gene expression data to guide drug development, with particular emphasis on microsatellite instable colorectal cancers.

Stephen B. Gruber is the H. Marvin Pollard Professor of Internal Medicine at the University of Michigan Medical School in Ann Arbor, USA, where he is also Professor of Internal Medicine, Human Genetics and Epidemiology. Before earning his medical degree at the University of Pennsylvania, PA, USA,, he earned an MPH with distinction from Yale University School of Medicine, New Haven, CT, and a PhD in epidemiology from Yale University. Following an internship and residency in internal medicine at the University of Pennsylvania, he completed a fellowship in medical oncology at John Hopkins Hospital in Baltimore, MD, and a fellowship in clinical medical genetics at the University of Michigan. His research interests include the molecular epidemiology and genetics of colorectal cancer, and the development of molecular markers for the clinical prediction of cancer risk.

Key points

- Approximately 15% of colorectal cancers (CRC) display microsatellite instability (MSI), and most are nonfamilial (sporadic) and caused by hypermethylation of the *MLH1* promoter
- 2–3% of all CRCs 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 dirty necrosis and the presence of a Crohn's 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
- 5-fluorouracil is the foundation of CRC therapy, but evidence suggests that this drug offers little benefit in early MSI CRC and higher response rates are achieved with irinotecan-based regimens
- 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

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 used 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.

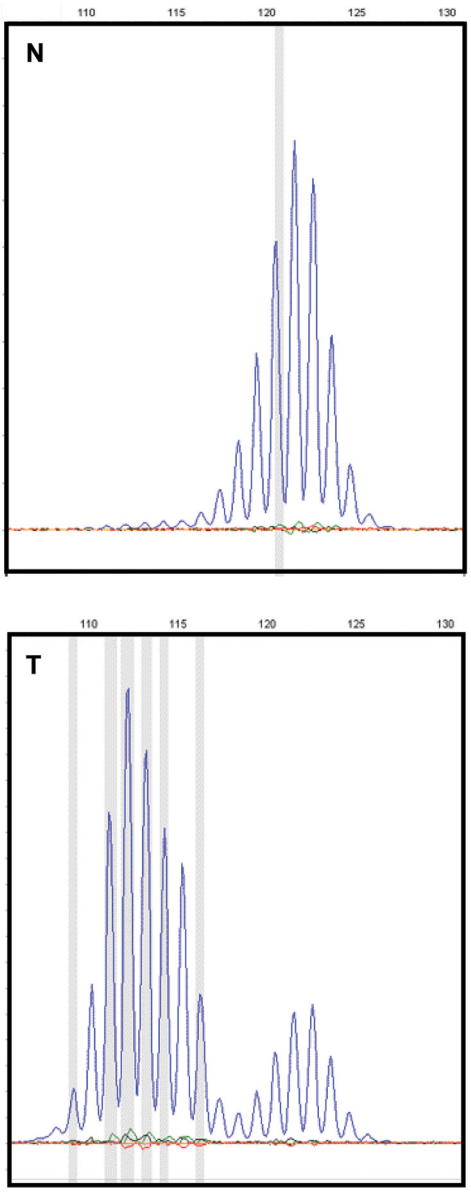

Figure 2.
Capillary electrophoresis of an MSI cancer. Electropherograms of the fluoresceinated amplification products for the loci BAT25 from normal colon mucosa (N) and colorectal cancer tissue (T). This loci contains $(A)_{25}$ repeats. Electropherograms can identify MSI CRC by the appearance of new shorter peaks owing to the shortening of the adenine repeats in cancer cells. The residual normal signal is under-represented with respect to the new unstable one, as most of the analyzed tissue is composed of neoplastic cells. Abbreviations: CRC, colorectal cancers; MSI, microsatellite instability.

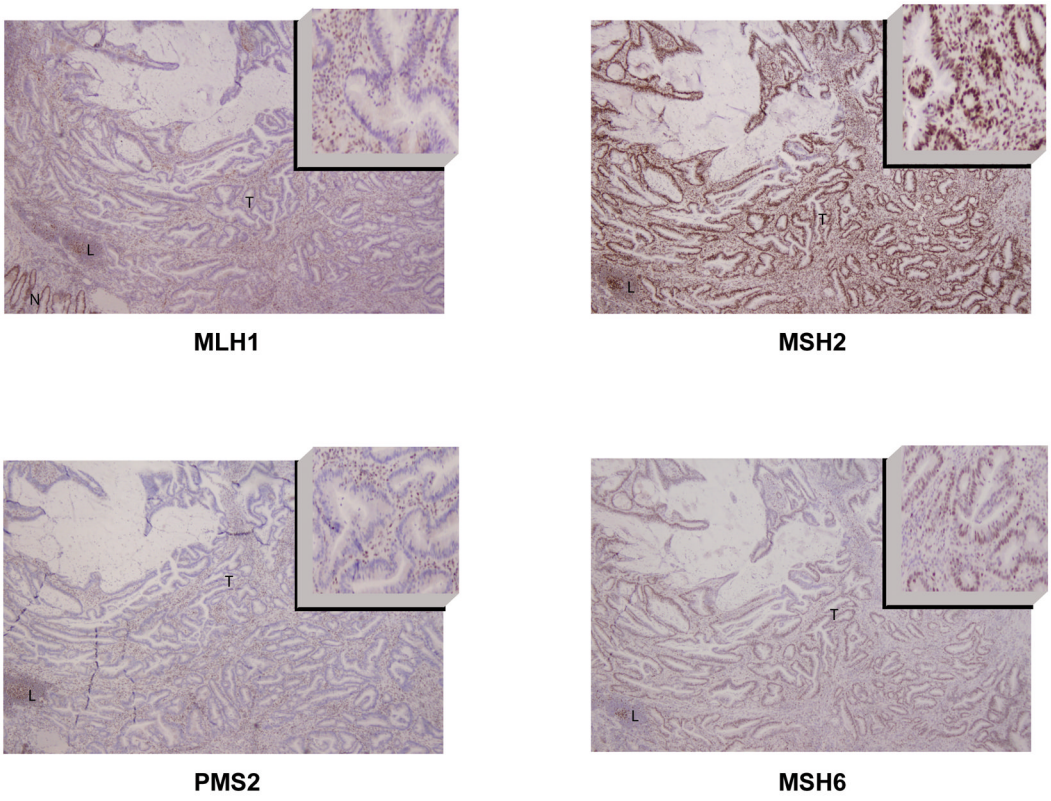

Figure 3.
Immunohistochemical patterns of mismatch repair proteins in colorectal cancer. Absent expression of a MLH1 and b PMS2, shown at X40 and X100 in tumor cells, with retained expression in normal colonocytes and lymphocytes. Intact expression of c MSH2 and d MSH6 shown at 40x and 100x in tumor cells and lymphocytes. Abbreviations: L, lymphocytes; T, tumor.

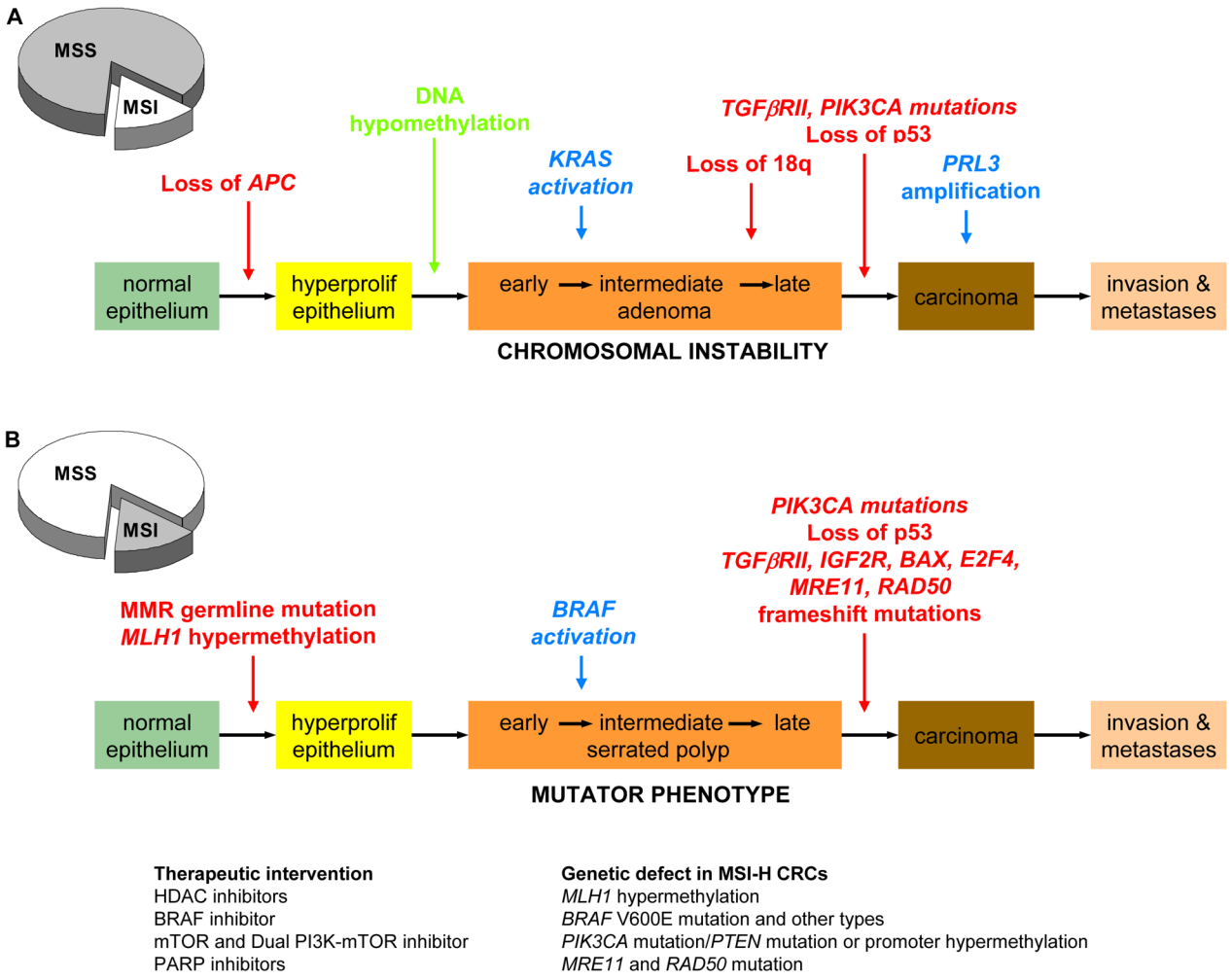

Figure 4.

Molecular CRC groups based on a chromosomal instability and b the mutator phenotype. The genetic models for CRC tumorigenesis are also presented in parallel to each pathway for tumor development. Target therapies based on molecular events are presented for MSI tumors.

Table 1

Interpretation of immunohistochemical patterns of mismatch repair deficiency

Protein				Interpretation	Inactivated gene	Microsatellite instability
MLH1	**MSH2**	**MSH6**	**PMS2**			
+	+	+	+	Intact MMR	None	MSS
+	−	−	+	Deficient MMR	*MSH2*[*]	MSI
+	+	−	+	Deficient MMR	*MSH6*	MSI or MSS
+	+	+	−	Deficient MMR	*PMS2*	MSI
−	+	+	−	Deficient MMR	*MLH1*[‡]	MSI

[*] Lack of expression of MSH2 and MSH6 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: MMR, mismatch repair; MSI, microsatellite instability; MSS, microsatellite stability.

Nat Rev Clin Oncol. Author manuscript; available in PMC 2012 August 24.

Table 2

Clinical studies analyzing microsatellite instability as a predictive marker of response to 5-FU-based chemotherapy
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.

Study	Study	Number of patients	Stage	MSI-H (%)	Durations of follow-up (months)	Stratification	5-FU effects	Primary end point	Chemotherapy regimen
Elsaleh *et al.*	R	656	III	8.5	54	MSI-H	Benefit	5 year OS	5FU+LEV
Hemminki *et al.*	P NR	95	III	12	31	MSI-H	Benefit	3 year DFS	5FU+FO 5FU+MTX 5FU+LEV 5FU
Liang *et al.*	P NR	244	IV	21.3	–	MSI-H	Benefit	mOS	5FU+FO high dose
Ribic *et al.*	R from RCT	570	II–III	16.7	88.8	ChT	Detriment	5 year OS/DFS	5FU+FO
Carethers *et al.*	R	204	II–III	17.6	43.7	ChT	None	mOS	5FU+LEV 5-FU based
Benatti *et al.*	R	1,263	All	20.3	64	ChT	None	5 year OS	5-FU based
Jover *et al.*	P NR	754	All	8.8	24.3	ChT	None	mDFS/mOS	5-FU based
Lamberti *et al.*	P NR	416	All	12.5	32.9	MSI-H	None	mOS	5-FU based
Kim *et al.*	R from RCT	542	II–III	18.1	60	ChT	None	mDFS/mOS	5-FU based
Sargent *et al.*	R from RCT	1,027	II–III	16	60	ChT	Detriment	mDFS/mOS	5FU+FO
Des Guetz *et al.*	MA	3,690	II–III	14		MSI-H	None	HR DFS/OS	5-FU based

Abbreviations: ChT, chemotherapy; DFS, disease-free survival; FO, folinic acid; 5-FU, 5-fluorouracil; HR, hazard ratio; LEV, Levamisol; 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; Strat, stratification.

Table 3

Gene expression studies characterizing microsatellite instable colorectal tumors and cell lines

Study	Expression platform	Number of MSI-H versus MSS	HNPCC	Gene classifier (*n*)	Molecular pathways
Koinuma *et al.*	Affy U133A&B	10/10	N	24	WNT, AXIN2, CTNNB1
Banerjea *et al.*	Affy U133A	27/104	Y	1,293	Immune response, Hsp-70, Hsp-110, interleukins, apoptosis
Kruhofer *et al.*	Affy U133A	34/67	Y	9	—
Watanabe *et al.*	Affy U133A&B	33/51	N	177	Apoptosis, inmune response, mucin
Giacomini *et al.*	Stanford Functional Genomics	8/10	Y	8	Metallotioneins
Vilar *et al.*	Affy Hu6800	13/38	Y	71	—

Abbreviations: Affy, Affymetrix; HNPCC, hereditary non-polyposis colorectal cancer; MSI-H, microsatellite instability-high; MSS, microsatellite stability