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REVIEW ARTICLE

Microsatellite instability in colorectal cancer

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Abstract

Approximately 20 percent of right-sided colon cancers and 5 percent of left-sided colon and rectal cancers have a deficient DNA mismatch repair system. This results in the widespread accumulation of mutations to nucleotide repeats, some of which occur within the coding regions of cancer-related genes such as *TGFβRII* and *BAX*. A standardized definition for microsatellite instability (MSI) based on the presence of deletions to mononucleotide repeats is gaining widespread acceptance in both research and the clinic. Colorectal cancer (CRC) with MSI are characterized histologically by an abundance of tumor-infiltrating lymphocytes, poor differentiation and a signet ring or mucinous phenotype. In younger patients these tumors usually develop along the chromosomal instability pathway, in which case the mismatch repair genes are inactivated by germline mutation, somatic mutation and loss of heterozygosity. In older patients MSI CRC usually develops against a background of widespread hypermethylation that includes methylation-induced silencing of the mismatch repair gene *MLH1*. The overall biological and clinical phenotype of MSI CRC that arise in these two pathways is likely to be different and may account for some of the discordant results reported in the literature relating to the clinical properties of these tumors. The available evidence indicates that MSI is unlikely to be a clinically useful marker for the prognostic stratification of early-stage CRC. The predictive value of MSI for response to 5-fluorouracil-based chemotherapy remains controversial, while for other agents the predictive value is difficult to assess because they are used in combination regimens. The MSI phenotype is being actively investigated for novel therapeutic approaches based on the principle of synthetic lethality. Finally, the MSI status of CRC is an extremely useful marker for population-based screening programs that aim to identify individuals and families with the hereditary cancer condition known as Lynch syndrome.

Key words: 5-FU, colorectal cancer, chemotherapy, Lynch syndrome, microsatellite instability, mismatch repair.

INTRODUCTION

Considerable progress has been made over the past 20 years in defining the molecular basis of colorectal cancer (CRC). The genetic heterogeneity of this disease is now well established and has major implications for studies on the etiology of CRC and its response to adjuvant therapy. One important subgroup of CRC is defined by widespread alterations in the size of DNA microsatellite regions, usually mono-, di-, or trinucleotide repeats. This so-called microsatellite instability (MSI) is due to a defective DNA mismatch repair (MMR) system and can result from inactivating mutations to MMR genes or, more commonly, because of hypermethylation-induced transcriptional silencing of the MMR gene *MLH1*. The underlying tumor phenotype between MSI CRC that arise from these two mechanisms is quite different. This fact has been largely overlooked by most workers in the field until very recently and could explain the discordant results in the literature relating to the predictive value of MSI. Although the MSI phenotype has yet to find routine clinical application as a prognostic or predictive marker,

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it has proven to be an extremely useful screening tool for the detection of families affected by hereditary nonpolyposis CRC, or Lynch syndrome. Moreover, the defective MMR system in MSI CRC could potentially be exploited for the development of novel targeted therapies. The purpose of this review is to summarize the current state of knowledge of the MSI phenotype in CRC, with particular emphasis on the detection of MSI for population-based screening of Lynch syndrome.

DISCOVERY OF MSI

MSI was first reported in 1993 as the presence of thousands of somatic alterations in the length of DNA microsatellite repeats in sporadic[1,2] and familial[3] colorectal tumors. Both mononucleotide and dinucleotide repeats display frequent somatic mutations in approximately 10–15 percent of sporadic CRC and in most familial cases. It was also reported that MSI CRC showed a lower incidence of *TP53* mutations and loss of heterozygosity (LOH), were more often diploid and often arose in the proximal colon. In the same year, the molecular basis for MSI in familial cases was discovered to be germline mutations in MMR genes, with the major ones being *MSH2, MLH1*, *PMS2,* and *MSH6*.[4,5] Inactivation of the remaining wild-type allele was through LOH, somatic mutation or methylation-induced silencing of *MLH1*. Despite many investigations, somatic mutations in MMR genes have rarely been found in sporadic MSI CRC and it later emerged that hypermethylation of the *MLH1* promoter region and subsequent transcriptional silencing was by far the most common mechanism leading to defective MMR in these tumors.[6]

The consequence of germline mutation, somatic mutation or methylation-associated silencing of MMR genes is inactivation of the DNA MMR process, thus leading to an accumulation of unrepaired alterations scattered throughout the genome. These are particularly prone to occur in DNA repeats such as microsatellites. The link between defective MMR and the development of CRC was first made in 1995 when somatic mutations in a 10 bp poly(A) repeat contained within the coding region of *TGF-βRII* were reported in CRC with MSI.[7] Following this discovery, many other target genes have since been found to be mutated in MSI tumors, including *BAX*, *TCF4*, *PTEN,* and *RAD50*.[8,9] These target genes all contain short repeat regions in their coding sequence, usually mononucleotide repeats of 8–10 bp in length, making them prone to mutation in tumor cells with defective MMR. The deletion or insertion of one or two nucleotides in these repeats causes a frameshift mutation resulting in the production of a truncated and therefore inactive protein. Target genes for MSI are involved in various critical cell functions including cell signaling, apoptosis, and DNA repair (Table 1). Their mutation is believed to drive the oncogenic process through positive selection. Target genes have also been identified by using bioinformatics to search for DNA coding microsatellites located within nucleotide sequence databases. In one study using this approach, 29 new genes that were mutated in MSI CRC were identified.[9] An interesting area that has received relatively little attention to date is the possibility that mutations to microsatellites located within non-coding regulatory regions (e.g. introns, promoters, 5′ and 3′ untranslated DNA) could affect gene expression in MSI CRC. So far, evidence in support of this has been published for the *MYB*[10] and *MRE11*[11] genes.

Table 1 Genes containing coding repeats that are targets for mutation in colorectal cancer with microsatellite instability

Function, *gene*	Mononucleotide coding repeat	Frequency of mutation (%)
DNA repair		
RAD50	(A) 9	28
MSH3	(A) 8	38
MSH6	(C) 8	22
BLM	(A) 9	9
Apoptosis		
APAF1	(A) 10	13
BAX	(G) 8	45
BCL10	(A) 8	13
Caspase-5	(A) 10	48
Signal transduction		
TGFβRII	(A) 10	81
ACTRII	(A) 8	58
IGFIIR	(G) 8	17
WISP-3	(A) 9	31
Cell cycle		
PTEN	(A) 6	18
RIZ	(A) 8, (A) 9	27
Transcription factor		
TCF-4	(A) 9	39

STANDARDIZING THE CLASSIFICATION OF MSI

The initial work on MSI focused on changes in the allelic size of both dinucleotide and mononucleotide repeats. An international consensus meeting was held at the National Cancer Institute (NCI) in 1997 with the aim of

formulating a standard panel of microsatellite markers that could be used to define MSI in CRC.[12] Despite some objections, the proposed NCI reference panel, also known as the Bethesda panel, contained two mononucleotide repeats (BAT25 and BAT26) and three dinucleotide repeats (D2S123, D17S250, and D5S346). It soon became apparent that allelic size shifts for the mononucleotide markers were relatively easy to interpret because the deletions observed in CRC with MSI were often large. In contrast, results obtained with the dinucleotide markers were frequently ambiguous and difficult to interpret because of the short length of insertions or deletions. Further complicating matters was the use of the term "MSI-L" to indicate a low level of MSI, defined by instability at only one of the five Bethesda reference markers. Subsequent comparative studies between the Bethesda panel and the pentaplex panel that comprised five mononucleotide repeats (described below) have shown this limitation was due to the inclusion of dinucleotide markers in the former panel, resulting in misclassification of MSI stable tumors into the MSI-L category.[13,14] Although CRC classified as MSI-L were phenotypically indistinguishable from microsatellite stable (MSS) CRC, defined by the absence of allelic shifts in any of the five markers,[15] this terminology is still in use by some authors.

The shortcomings associated with the original Bethesda panel of five markers proposed for MSI detection were highlighted by Perucho *et al.*[16] Moreover, the work of Hamelin *et al.* clearly demonstrated the superiority of mononucleotide repeats over dinucleotide markers for the assessment of MSI, particularly the BAT26 poly(A) repeat.[17,18] First, deletions in mononucleotide repeats were longer and therefore easier to detect than alterations in the size of dinucleotide repeats. Second, the mononucleotide repeats were quasi-monomorphic, meaning that normal tissue was not required for MSI testing in most cases. However, the use of BAT26 alone is not recommended for diagnostic MSI screening because of the existence of polymorphisms in approximately 10 percent of the African population that can lead to false positive results for MSI.[19] In addition, very rare cases of bi-allelic deletion of the BAT26 locus can result in false negative reporting for MSI.[20]

For these reasons, a comprehensive study was undertaken by Hamelin *et al.* to investigate polymorphisms in several candidate mononucleotide repeats and the frequency and extent of deletions in these repeats in MSI tumors.[21] This landmark article proposed a five-marker, or pentaplex panel for MSI screening that comprises the mononucleotide repeats BAT25, BAT26, NR21, NR22, and NR24. The pentaplex panel showed 100 percent sensitivity and 100 percent specificity for the detection of MSI and can be used without the need to test matching normal DNA, although most routine laboratories include normal tissue DNA to assist with the interpretation of results. The pentaplex assay is commercially available and has been used by routine anatomical pathology laboratories for several years. An example of the results obtained with this assay in a routine clinical specimen of CRC from our institute is shown in Figure 1. Several independent studies have found the pentaplex panel performs better in terms of sensitivity and specificity than the original NCI panel containing three dinucleotide and two mononucleotide repeats and

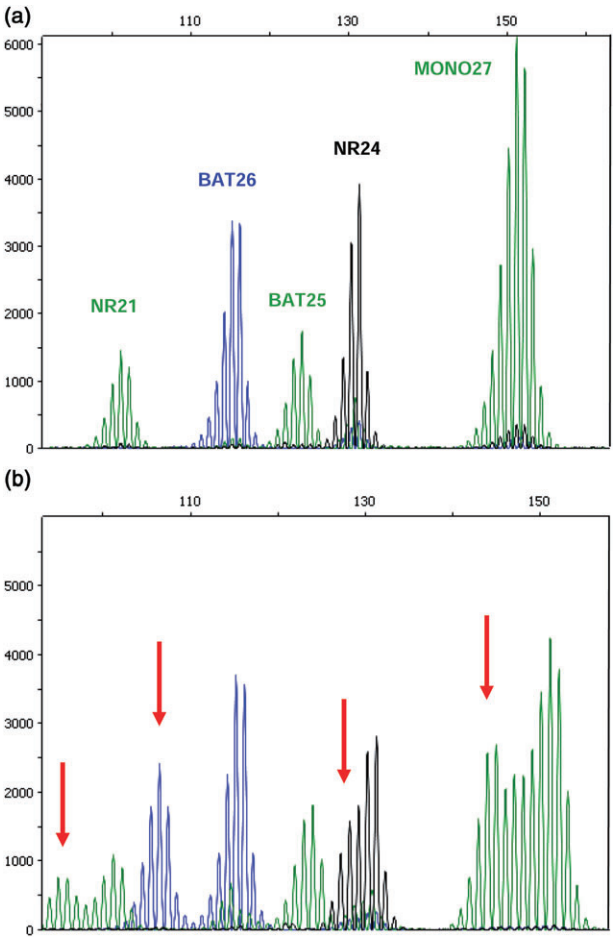

Figure 1 Screening for microsatellite instability in a clinical sample of colorectal cancer using the pentaplex panel consisting of five mononucleotide repeats showing (a) normal tissue in comparison to (b) additional bands in matching tumor tissue (arrowed), indicating deletions in four of the repeats (NR22, BAT26, NR24, and MONO27).

can accurately reclassify MSI-L cases identified with the Bethesda panel into MSI-High or MSS tumors.[13,14,22] The simultaneous assessment of just two markers, BAT26 and NR24, was shown to be as effective as the pentaplex panel for the diagnosis of MSI.[22]

THE MSI PHENOTYPE IN CRC CAN EVOLVE THROUGH TWO DIFFERENT PATHWAYS

The methodological issues surrounding the evaluation of MSI have resulted in considerable variation in the reported frequencies of MSI in CRC, with estimates ranging from 3–23 percent.[15] The true incidence of MSI CRC as observed in large studies of unselected tumor series that employed mononucleotide repeat markers is approximately 10–15 percent.[23–25] Overestimation of the MSI frequency resulting from the use of dinucleotide markers has led to confusion regarding the clinico-pathological features of MSI CRC.[16] When mononucleotide markers are used for the evaluation of MSI, consistent observations are that sporadic MSI CRC arise almost exclusively in the proximal colon and are more frequent in older, female patients.[26] Characteristic histo-morphological features include dense lymphocytic infiltration, mucin secretion and poor histological differentiation.[27] Although these morphological features are common to both sporadic and familial MSI CRC, there is now strong evidence these two MSI subgroups evolve along different pathways.[27,28]

Most sporadic MSI CRC arise due to methylation-induced transcriptional silencing of *MLH1*. This hypermethylation occurs as part of a wider CpG island methylator phenotype (CIMP) pathway.[29] In contrast, familial MSI CRC that arise in the context of Lynch syndrome follow the chromosomal instability phenotype (CIN) pathway, characterized by frequent LOH, aneuploidy and *TP53* mutation. The discovery of the existence of two types of MSI that evolve along either the CIMP or CIN pathways has major implications for studies that attempt to define the clinico-pathological features of MSI CRC. This has been largely ignored by most studies to date on the etiology of MSI CRC and on the prognostic and predictive significance of this molecular phenotype.[30,31]

In a population-based cohort of 1020 consecutive CRC, MSI was more common in male patients aged <60 years, equally common in male and female patients aged 60–70 years, but threefold to fourfold more common in female patients aged >70 years.[32] This distribution reflects the association of gender and age with the CIN and CIMP phenotypes. CRC with the CIN phenotype are more common in younger men, whereas CIMP CRC are more common in older women. Another large study that compared 1061 population-based CRC with 172 CRC diagnosed at a familial cancer clinic also highlights the striking age and gender differences in *MLH1* methylation and MMR mutation status between MSI cases that are sporadic or familial in origin.[31]

IS MSI A PROGNOSTIC MARKER IN CRC?

Robust prognostic markers are particularly desirable for early stage CRC so that informed decisions can be made on the possible benefits of adjuvant chemotherapy. Although numerous studies have investigated the relationship between MSI and the survival of CRC patients, the results are often difficult to interpret because of several confounding factors. First, almost all MSI CRC study cohorts are a heterogeneous mix of CIN and CIMP subtypes. As discussed above, there is increasing evidence to show these have different clinical properties. Second, the study cohorts are almost always composed of tumors of different stages. Since most MSI CRC are early stage (American Joint Committee on Cancer stage I or II), this could explain the apparently better survival reported in many studies. In support of this, a study of 893 consecutive cases found that MSI did not predict a lower risk of cancer-related death when tumor stage was included in a multivariate analysis.[25] Another study of 396 consecutive cases of stage II CRC also failed to observe prognostic significance for MSI.[33] Third, the possibility of a differential response to chemotherapy between MSI and MSS tumors (see below) complicates the interpretation of results. Adjuvant treatment status has not been taken into account in most studies. Finally, many of the early studies used the less rigorous dinucleotide markers to classify MSI status and are likely to have overestimated the presence of true MSI.

Notwithstanding these caveats, a systematic review of 32 studies comprising a total of 7642 cases, including 1277 that were MSI, reported a hazard ratio (HR) of 0.65 (95% *CI:* 0.59–0.71) for overall survival associated with MSI.[34] However, the lack of convincing data showing that MSI is an independent marker for better survival in stage II CRC raises serious doubts about whether it should be used clinically for prognostication. In our experience and that of other groups, the routinely assessed histopathological features of vascular and serosal invasion by tumor cells, together with the density

of tumor-infiltrating T regulatory cells, provide much stronger prognostic information than MSI.[35–38]

IS MSI A PREDICTIVE MARKER IN CRC?

The possible impact of MSI as a predictive factor in the response of CRC to chemotherapy has been extensively studied both in *in vitro* and in clinical samples. Similar to work on the prognostic value of MSI, these studies are complicated by the issue of whether MSI arises in the background of the CIN phenotype, as in most young and familial cases, or whether it arises in a CIMP background, as occurs in most sporadic cases. The presence of these underlying phenotypes could be more important for the response to cytotoxic drugs than the MMR deficient phenotype itself and may account for the contradictory findings on the sensitivity of MSI CRC cell lines to the fluorinated pyrimidine analog 5-FU.[39,40] Indeed, *in vitro* data that are claimed to support a differential response of MSI tumor cells to cytotoxic agents is likely to be highly dependent on the cell lines used.

Clinical studies on the predictive value of MSI for survival benefit from 5-FU chemotherapy in CRC patients have also produced contradictory results. Apart from the CIN and CIMP heterogeneity of MSI CRC, another major issue relates to the 10-fold lower frequency of MSI CRC in comparison to MSS CRC. Statistical power to detect a survival difference between patients treated with or without chemotherapy is therefore considerably lower for MSI tumors. The first clinical study was conducted in a retrospective cohort of patients and reported that MSI was associated with good survival benefit from 5-FU-based chemotherapy in stage III CRC.[41] A subsequent study performed in a young cohort of patients, many of whom were likely to be familial cases, found no apparent survival benefit from 5-FU.[42] Several other studies performed on retrospective patient cohorts have since been published and their results, together with those of the two earlier studies, were recently the subject of a meta-analysis.[43] MSI was associated with survival benefit from chemotherapy in a global analysis of the six studies evaluated, although this failed to reach significance (HR = 0.70; 95% *CI:* 0.44–1.09; $P = 0.12$). Interestingly, the HR from the individual studies were 0.61,[44] 0.60,[45] 0.49,[46] 0.22,[47] and 0.07, respectively.[41] Only the study by Ribic *et al.*[42] showed a grossly different result (HR = 2.38) and was in fact suggestive of worse survival for MSI CRC patients following 5-FU treatment. Based largely on the results of the study by Ribic *et al.*[42] several authors have proposed that CRC patients with MSI tumors should not be recommended to receive adjuvant chemotherapy.[48,49] A possible explanation for the discrepant result reported in the study by Ribic *et al.*[42] could be that most MSI tumors from their patient cohort had a CIN background, whereas most MSI tumors in other studies were from older patients and therefore had a CIMP background. The only study published to date on the predictive significance of CIMP in CRC suggests this phenotype is associated with a good response to 5-FU.[50]

The authors of the Ribic *et al.* study recently published a follow-up study on a cohort of 70 MSI patients.[51] Adjuvant therapy was found to have significantly improved survival in patients with MSS tumors (HR = 0.67; $P = 0.02$), but not in those with MSI tumors (HR = 1.10; $P = 0.85$). In the subgroup of patients with stage II disease and MSI tumors, treatment was associated with an apparent reduction in overall survival (HR = 2.95; $P = 0.04$). No explanation was offered as to why chemotherapy is detrimental to the survival of such patients. In an accompanying editorial to this article,[52] Schrag *et al.* argue that adjuvant 5-FU-based chemotherapy should not be withheld from stage II and III colon cancer patients with MSI tumors, citing the need for prospective evaluation and the heterogeneity of MSI tumors (CIN and CIMP backgrounds).

Several *in vitro* studies have shown the MSI phenotype to be associated with resistance to cisplatin and carboplatin, but not to oxaliplatin.[53,54] However, a recent clinical study reported that MSI CRC were less responsive to 5-FU and oxaliplatin.[55] These contradictory findings suggest it is premature to use MSI status to direct the use of oxaliplatin-containing regimens for CRC patients. The topoisomerase I inhibitor, irinotecan, is also widely used in the treatment of metastatic CRC. Most of the *in vitro*, xenograft and clinical studies to date indicate that MSI CRC are more sensitive than MSS CRC to irinotecan.[56–60] Although the molecular basis for this increased sensitivity is not fully understood, there is evidence to suggest it involves mutations within coding repeats of two of the frequently mutated target genes, *MRE11A* and *RAD50*.[61]

No information is currently available on the predictive value of MSI for the anti-epidermal growth factor receptor (EGFR) and anti-vascular endothelial growth factor targeted therapies. In practice, the clinical value of MSI for predicting the response of metastatic CRC to chemotherapeutic agents is almost impossible to evaluate due to the low incidence of MSI in advanced stage tumors (<5%) and because most agents are now given in combination.

DEFECTIVE MMR AS A NOVEL THERAPEUTIC TARGET

High-throughput array technology, bioinformatics and a systems biology approach are increasingly being used to identify aberrant genes and pathways that may serve as targets for inhibition in cancer cells. One such study recently found that MSI CRC cells were sensitive to inhibitors of the PI3K-AKT-mTOR pathway,[62] although the clinical significance of this finding awaits confirmation. Another recent therapeutic strategy is to identify synthetic lethal relationships, where the simultaneous inhibition of two different regulatory pathways leads to cell death.[63] This approach has been used successfully in the clinic for the treatment of BRCA-1-deficient and BRCA-2-deficient breast and ovarian cancers with poly (ADP-ribose) polymerase (PARP) inhibitors.[64] CRC with deficient MMR may also be suited to this strategy, although preliminary *in vitro* data failed to show a differential response to PARP inhibitors between MSI and MSS CRC cell lines.[65] A synthetic lethal relationship has been found between MSH2 deficiency and treatment with methotrexate,[66] giving rise to a phase II trial in metastatic CRC patients with demonstrated loss of MSH2 expression or with an *MSH2* germline mutation. Yet another approach for the selective treatment of MSI CRC is to exploit the chromosomal stability of MSI tumors, which is predicted to increase their sensitivity to taxanes. The chromosomal instability and anti-tubulin response assessment (CINATRA) trial has been initiated to test whether MSI tumors are more responsive to patupilone, a novel microtubule-stabilizing agent.[67]

ROUTINE MSI SCREENING FOR THE DETECTION OF LYNCH SYNDROME

Although both the prognostic and predictive significance of MSI in routine clinical practice are still being debated, there is strong evidence to support the value of this marker for the detection of families with hereditary nonpolyposis colorectal cancer, now more commonly referred to as Lynch syndrome. This familial cancer syndrome has been estimated to account for 1–2 percent of all CRC cases in the population (Table 2).[68–75] The young age of disease onset in mutation carriers (average of <45 years) highlights the importance of identifying these individuals so that they and other affected family members can benefit from increased surveillance and early cancer detection. Until recently, the clinical factors of young age and family history of cancer were used to identify patients for referral to specialized family cancer clinics where they could undergo further evaluation and possible germline testing. However, the low rates of referral by clinicians to these clinics and the low rates of attendance by patients[76] have led to concerns that as many as 80 percent of mutation carriers in the population remain undetected.[77–79] This, in turn, has led to calls for the establishment of population-based screening programs based upon the laboratory tests of MSI or immunohistochemistry (IHC), or both, for MMR protein expression.[79]

Table 2 Population-based studies that used microsatellite instability as the initial screening test to detect cases of Lynch syndrome

Author	Country	*N*	Frequency of Lynch syndrome (%)
Aaltonen[68]	Finland	509	2.0
Salovaara[69]	Finland	535	3.4
Samowitz[70]	USA	1066	0.86
Percesepe[71]	Italy	336	0.3
Cunningham[72]	USA	257	1.9
Pinol[73]	Spain	1222	0.9
Hampel[74]	USA	1066	2.2
Schofield[75]	Australia	1344†	0.83

†Consecutive colorectal cancer cases from patients aged <60 years at diagnosis.

IHC tests for expression of the four major MMR proteins (MLH1, MSH2, PMS2, and MSH6) have been the preferred option by routine anatomical pathology laboratories because of their long experience with this technique. The test is relatively straightforward to perform and the demonstration of complete loss for the MLH1-PMS2 or MSH2-MSH6 protein pairs is an accurate indicator of deficient MMR and MSI. The surrounding normal colonic mucosa and stromal tissue serves as internal positive controls. Cases that exhibit incomplete loss of expression, also described as clonal or heterogeneous expression, are more difficult to interpret. In these instances it is strongly recommended that MSI analysis should be conducted to assist with the interpretation.[80] Results from our laboratory and others have shown that tumors from patients with MMR germline mutations can sometimes show incomplete loss of MMR protein expression,[80–82] potentially leading to an incorrect diagnosis. For this reason, our laboratory favors MSI testing as the initial screen for population-based detection of Lynch syndrome amongst younger CRC patients.[75]

All the large population-based studies published to date for the screening of Lynch syndrome have used MSI

as the initial screen, rather than IHC (Table 2). This approach has yet to be widely adopted, however, due to the relatively recent introduction of molecular testing in routine pathology laboratories. In parallel with the need for MSI testing, there are increasing demands for polymerase chain reaction-based screening of somatic mutations in *KRAS*, *BRAF,* and *EGFR*, for example, relating to the use of novel targeted therapies. Most of the larger pathology service providers have now gained valuable experience in the molecular testing of solid tumors, often using archival, paraffin-embedded tumor tissues. This advancement of technical expertise should facilitate the implementation of routine MSI testing in CRC. Aside from being a biomarker for responses to certain targeted therapies, *BRAF* mutation status is also a very useful tool to distinguish between cases of familial and sporadic MSI CRC.[83,84]

The proportion of MSI CRC with an underlying germline MMR gene mutation has been estimated to decrease from 80–90 percent for <40-year-old patients to 68 percent for 40–49-year-old patients and to 17 percent for 50–59-year-old patients.[75] While most CRC in mutation carriers arise before the age of 60 years, some do occur in older patients.[85] This has led to debate concerning the recommended cut-off age for population-based MSI screening.[85,86] Ideally, all CRC patients would be tested for MSI, *BRAF* mutation, and IHC, regardless of family history of cancer. So-called red flag cases showing MSI, *BRAF* wild type, and loss of MMR expression would then be referred to familial cancer clinics for germline testing. In practice, however, budget constraints and the lack of expertise for MSI and *BRAF* testing in many routine pathology services will restrict the ability to roll out population-based screening for Lynch syndrome.

CONCLUSIONS

MSI is a distinctive molecular phenotype seen in about 15 percent of sporadic CRC and in almost all tumors from individuals with Lynch syndrome. Early studies of this phenotype were confounded by the lack of a standardized panel of markers, the slow recognition of mononucleotide repeats as the most suitable markers and the use of MSI-L terminology. A pentaplex panel comprising five mononucleotide repeats is gradually finding widespread use as the gold standard for the evaluation of MSI status. However, a major issue that has received little attention to date is the fact that MSI tumors can develop along either the CIN or CIMP pathways of CRC. The overall phenotype of these tumors may therefore be influenced more by their underlying CIN or CIMP background than by the MSI itself. This biological heterogeneity has not been considered in most studies to date on the prognostic and predictive significance of MSI in CRC. Although MSI appears to be associated with better prognosis, questions about the strength and independence of this association mean that it is unlikely to be of clinical value for the stratification of early-stage CRC patients. The predictive significance of MSI for a response to 5-FU remains controversial and more work is required to determine whether the CIN and CIMP subgroups show differential responses. The predictive significance of MSI for other chemotherapeutic agents is difficult to assess because these are usually given in combination. Deficient MMR in tumors with MSI may serve as a target for novel therapies based upon synthetic lethal strategies. Finally, MSI is a very useful marker for population-based screening of CRC patients to help identify individuals and families with Lynch syndrome.

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