Complementary analysis of microsatellite tumor profile and mismatch repair defects in colorectal carcinomas

Alfredo Blanes, Salvador J Diaz-Cano

Abstract

Microsatellite instability (MSI) is a prognostic factor and a marker of deficient mismatch repair (MMR) in colorectal adenocarcinomas (CRC). However, a proper application of this marker requires understanding the following: (1) The MSI concept: The PCR approach must amplify the correct locus and accurately identify the microsatellite pattern in the patient’s normal tissue. MSI is demonstrated when the length of DNA sequences in a tumor differs from that of nontumor tissue. Any anomalous expansion or reduction of tandem repeats results in extra-bands normally located in the expected size range (100 bp, above or below the expected product), differ from the germline pattern by some multiple of the repeating unit, and must show appropriate stutter. (2) MSI mechanisms: MMR gene inactivation (by either mutation or protein down-regulation as frequently present in deep CRC compartments) leads to mutation accumulation in a cell with every cellular division, resulting in malignant transformation. These mechanisms can express tumor progression and result in a decreased prevalence of aneuploid cells and loss of the physiologic cell kinetic correlations in the deep CRC compartments. MSI molecular mechanisms are not necessarily independent from chromosomal instability and may coexist in a given CRC. (3) Because of intratumoural heterogeneity, at least two samples from each CRC should be screened, preferably from the superficial (tumor cells above the muscularis propria) and deep (tumor cells infiltrating the muscularis propria) CRC compartments to cover the topographic tumor heterogeneity. (4) Pathologists play a critical role in identifying microsatellite-unstable CRC, such as occur in young patients with synchronous or metachronous tumors or with tumors showing classic histologic features. In these cases, MSI testing and/or MMR immunohistochemistry are advisable, along with gene sequencing and genetic counseling if appropriate. MSI is an excellent functional and prognostically useful marker, whereas MMR immunohistochemistry can guide gene sequencing.

© 2006 The WJG Press. All rights reserved.

Key words: Colon carcinoma; Microsatellites; Mismatch Repair; Hereditary non-polyposis colon cancer

Blanes A, Diaz-Cano SJ. Complementary analysis of microsatellite tumor profile and mismatch repair defects in colorectal carcinomas. World J Gastroenterol 2006; 12(37): 0000-0000

http://www.wjgnet.com/1007-9327/12/.asp

INTRODUCTION

Colorectal carcinoma (CRC) is generally classified into three categories, based on increasing hereditary influence and cancer risk\[1,9\]: sporadic CRC (approximately 60% of cases and comprises patients with no notable family history and, by definition, with no identifiable inherited gene mutation that accelerates cancer development), familial CRC (approximately 30% of cases and refers to patients who have at least one blood relative with CRC or an adenoma, but with no specific germline mutation or clear pattern of inheritance), and hereditary CRC syndromes (approximately 10% of cases, which result from inheritance of a single gene mutation in highly penetrant cancer susceptibility genes). Although the last group has the lowest frequency, it has elucidated molecular mechanisms of carcinogenesis applicable to sporadic CRC\[10\].

The microsatellite profile of sporadic CRC is a prognostically useful marker\[4-7\]. Microsatellites are repeating DNA sequences of unknown function that are found throughout the genome\[11\]. Microsatellite instability (MSI) is demonstrated when the length of DNA sequences in tumor and nontumor tissues is different and MSI has been identified in a wide variety of human tumors, due to defects in one of the DNA mismatch repair (MMR) genes, especially MLH1 or MSH2\[12,13\]. However, MSI presence alone does not establish a diagnosis of hereditary non-polyposis colon cancer (HNPCC) because MSI has also been identified in 10%-30% of sporadic CRC. Certain histological features also correlate with the presence of
MSI in sporadic CRC (Figure 1)\textsuperscript{[5,10-13]}, which can be key elements in the design of more effective therapeutic protocols\textsuperscript{[12,13]}.

Both basic and clinical implications of MSI and MMR defects need to be considered in an appropriate context, which requires clarifying the definition of MSI, the biological consequences of tumor MSI, interference of intratumor heterogeneity on MSI detection, differences in clinical testing for MSI and for MMR defects, and MSI prognostic and therapeutic implications.

**MSI DEFINITION AND CLINICAL TESTING FOR MSI**

Any useful application of prognostic factors requires a reliable definition of the factor. Microsatellites belong to the family of highly polymorphic and repetitive non-coding DNA sequences that, although widely distributed in the human genome, are not uniformly spaced (underrepresented in subtelomeric chromosome regions). Microsatellites are useful molecular markers due to their...
ubiquity, PCR typability (except for (dA)n multimers, whose size polymorphisms are difficult to type), Mendelian co-dominant inheritance, and extreme polymorphism[10], but their origin and function are not clear[14]. They have been demonstrated to be very useful in cell lineage delineation, positional cloning, and several applications in forensic medicine[15,16]. Microsatellite instability (MSI) is demonstrated when the lengths of DNA sequences in a tumor differ from those of nontumor tissue. MSI has been identified in a wide variety of human tumors.

Currently, tumor MSI analyses require molecular tests and the application of strict criteria. MSI can be defined as a change in any DNA sequence length due to either insertion or deletion of repeating units in a microsatellite within a tumor when compared to normal tissue[17,18]. The tests must be run with appropriate controls (known positive and negative controls along with the patient’s normal tissue)[19,20], which are extremely important due to the non-exceptional presence of extra-bands. The PCR approach must amplify the correct locus and accurately identify the microsatellite pattern in the patient’s normal tissue. Any anomalous expansion or reduction of tandem repeats due to MSI results in extra-bands. True extra-bands expressing tumor MSI are normally located in the expected size range (usually about 100 bp), are above or below the expected PCR product, differ from the germline pattern by some multiple of the repeating unit (e.g. delta 6 bases for dinucleotides), must show appropriate stutter (e.g. -2, -4 for dinucleotides), and are not present in the normal control. These tests should be carefully analyzed considering the following: (1) Sample homogeneity/heterogeneity can vary. Very small samples (even single cells) have been used in genetic analyses to avoid normal cell contamination. However, the lower the number of cells the higher the probability of technically-related abnormal results[20], which can be partially resolved with appropriate methods. The high incidence of PCR artifacts using microdissected samples is related to the small concentration of target DNA, fixation induced changes of DNA, and conditions in the amplification of repetitive sequences (especially for those CG-rich sequences) favouring misannealing and hairpin formation. Appropriate modifications to avoid the above conditions will significantly improve the reproducibility of LOH and MSI tests in microdissected samples[21]. (2) Appropriate controls are necessary for every step of the molecular tests to avoid false results. Sufficient levels of amplification with all markers should be obtained to detect low amounts of shifted microsatellites. (3) PCR bias against one allele (especially the larger one in a pair) can result in preferential amplification of the other allele (usually the smaller in a pair), which is the so-called artifactual allele dropout[22,23]. An appropriate extraction method, providing DNA of quality[24,25], and PCR designs including both long denaturing and extension in the first three cycles and 7-deaza-dGTP in the amplification mixture to improve the amplification of CG-rich DNA regions, will be reasonably helpful in avoiding that bias[8,9,10,12,20,28]. (4) The number of polymorphic DNA regions agreed to at the NCI consensus conference includes a primary panel of at least 2 mononucleotide and 3 dinucleotide microsatellites, along with 19 alternate loci (both mono- and dinucleotides)[29]. The choice of microsatellite markers is important in MSI testing, with the examination of mononucleotide repeats being sufficient for detection of MMR deficient tumors, whereas instability only in dinucleotides is characteristic of MSI-L/MMR-positive tumors[30]. Depending on the number of abnormal loci from the total analyzed, the cases are classified into MSI-high (≥ 30%-40% of abnormal loci), MSI-low (< 30%-40% of abnormal loci), and MSS (no abnormal loci).

Which patients should be tested? The neoplasm histological features closely correlate with MSI and should be the key elements used to select sporadic CRC for MSI investigation[5,10-13]. The sets of criteria for the clinical diagnosis of HNPCC appear under Clinical Testing for MMR defects. The implications of these analyses in sporadic and HNPCC carcinomas are compared in Table 1.

| Table 1 False negative in antigen positive neoplasms, comparative features of microsatellite unstable sporadic adenocarcinoma and hnpcc colon carcinomas |
|-----------------------------------------------|------------------|
| MSI-H | Sporadic Adenocarcinomas | HNPCC Adenocarcinomas |
| Patient age | Older | Younger |
| Number of tumors | Single | Single/Multiple (synchronous/metachronic) |
| Colonic distribution | Right colon | Right colon |
| Histological clues | Poorly differentiated, medullary type | Poorly differentiated, medullary type |
| | Crohn-like inflammation | Crohn-like inflammation |
| Mechanism of MMR deficiency | MLH1 promoter hypermethylation | Inactivating germline mutation of MMR proteins |
| Tumor prognosis | Better than MSI-L/ MSS sporadic adenocarcinoma | Better than MSI-L/ MSS sporadic adenocarcinoma |

**BIOLGICAL CONSEQUENCES OF TUMOR MSI**

Microsatellite-unstable CRC are biologically different and have a better survival rate than sporadic CRC when matched for cancer stage[28-30]. The development of proximal and distal CRC involves partly different mechanisms associated with the MSI and the chromosomal instability (CIN) pathways[31].

These two pathways are not always independent and some CRCs show a significant degree of overlap between these two mechanisms[32]. In one study, 35% of CRC were microsatellite-unstable (21% MSI-low and 14% were MSI-high) and 51% of CRC had at least one LOH event, with the most frequent chromosomal losses observed on 18q (72.5%)[32]. A significant degree of overlap between MSI and CIN pathways has been reported in that series: 6.5% of CRC with LOH were also MSI-high, and 23.3% of MSI-high CRC also had one or more LOH events. These data suggest that molecular mechanisms of genomic instability are not necessarily independent and may not
be fully defined by either the MSI or CIN pathways. In addition, a subgroup of CRCs showed no evidence of alterations in either of these two pathways of genomic instability (37.8% of microsatellite-stable CRCs had no LOH events identified)\(^{[25]}\), a situation similar to that reported in muscle-invasive transitional cell carcinomas of the bladder\(^{[25]}\).

MMR proteins normally identify and correct mismatched DNA sequences that can occur during DNA replication. An inactivating mutation in any of these genes leads to mutation accumulation in a cell with every cellular division, resulting in malignant transformation\(^{[6,8,33-36]}\). Tumor progression in the deep compartments may be the result of MMR protein down-regulation, which would contribute to the following: (1) There is a decreased prevalence of aneuploid cell lines and K-RAS and B-RAF mutations detected in microsatellite-unstable CRC and in the deep compartments of sporadic CRC\(^{[7,29,37,38]}\). Microsatellite-unstable CRCs tend to be diploid\(^{[37,39]}\), and to have lower DNA indices\(^{[39]}\). Supporting these findings, the MMR protein down-regulation observed in the deep compartments of sporadic CRC has shown correlation with increased frequency of diploid DNA content\(^{[40,41]}\). (2) Differential cell kinetics (proliferation and apoptosis) has been identified in superficial and deep compartments (above muscularis propria vs. muscularis propria) of sporadic CRC, which has demonstrated a close correlation with MMR protein expression (Figure 2)\(^{[41,42]}\). Physiologic correlations between MMR protein expression and kinetic variables (mitotic figures, Ki-67 expression, ISEL index) were preserved in the superficial compartment only. In addition, G2 + M phase fraction correlated with hMLH1 expression only in superficial compartments and hMSH2 expression only in deep compartments. Both the high cellular turnover and the maintained cell kinetic balance suggest that superficial compartments of sporadic CRC are expansible. In the deep compartments, the expression of MMR proteins is inefficient (not correlated with G2 + M phase fraction) and is dissociated (only one gene product correlates with G2 + M), which would eventually result in mutation accumulation and progression\(^{[40]}\).

**INTRATUMOR HETEROGENEITY AND MICRO-SATELLITE ANALYSIS**

Tumor cell heterogeneity is linked to genetic instability and biologic progression. This problem must be studied by including several tumor samples of sufficient size from each tumor.

The sample size is an important parameter. Microdissection techniques allow selectively picking up very small samples, which can show false cellular homogeneity, based on the loss of heterozygosity or allelic imbalance. If the tumor cell populations selected for molecular analysis are taken before they become a biologically prominent component (with kinetic or invasive advantages), the results might be confusing and clinically non-relevant. This would be a case of tumor microheterogeneity, which tends to give disparate results with meanings essentially unknown. Except for intraepithelial proliferation, all microdissected cell samples provide target cell-rich samples with varying degrees of host cell contamination (including stromal, inflammatory, and endothelial cells). Therefore, multiple samples from the same case should always be studied and assays performed in duplicate before accepting the results as relevant.

The intratumor heterogeneity can result in discordant results for a given marker depending on the sample origin. The comparison of MMR protein expression and PCR-based MSI studies has revealed discordant results in 8% of right-sided sporadic CRC and complete concordance after performing further analyses on other tumor areas\(^{[39]}\). Because of this intratumor heterogeneity, at least two samples from each CRC should be screened, although no systematic approach has been used to address this topic in sporadic CRC. Microsatellite analysis in muscle-invasive transitional cell carcinomas of the bladder have revealed topographic heterogeneity in 32% of cases, showing that the deep compartment had more microsatellite abnormalities (20%)\(^{[21]}\). We have found significant differences between superficial (tumor cells above the muscularis propria) and the deep (tumor cells infiltrating the muscularis propria) compartments of sporadic CRC, the deep compartments showing MMR protein down-regulation and increased MSI\(^{[41,44]}\). At least one-third of unstable tumors in deep compartments can be expected to be stable in superficial compartments. These differences can eventually result in the classification of a given tumor as MMS or MSI depending on the sample origin (superficial or deep).

**CLINICAL TESTING FOR MMR DEFECTS**

MSI results from the dysfunction of MMR proteins, which can be detected at genetic or protein levels. It is recommended that a CRC should be tested for MSI prior to gene testing, since this test is inexpensive and will help predict whether or not an individual has a germline MMR gene mutation\(^{[45,46]}\). Since up to 5% of HNPCC tumors do not have MSI, negative MSI tests cannot completely rule out HNPCC. Conversely, a positive MSI test is not diagnostic of HNPCC because 15%-30% of unselected CRC have MSI (due to MLH1 promoter methylation), whereas only 1%-6% of all CRC are associated with detectable HNPCC mutations. If the tumor is MSI-positive, further analyses for MMR defects are recommended.

**Genetic testing for MMR defects**

MMR defects are due to either inactivating point mutations spread throughout the genes, therefore needing full-length sequencing, or promoter hypermethylation (especially MLH1 in sporadic CRC). HNPCC is an autosomal dominant disorder caused by germline MMR gene mutations, in particular in MLH1, MSH2, MSH6, and PMS2. No strong genotype-phenotype correlations have been observed to date, but MSH2 mutations do appear to be associated with more extracolonic manifestations than MLH1 mutations. MSH6 mutations are more common in endometrial tumors and PMS2 mutations are especially
associated with Turcot's syndrome\(^\text{[17]}\). The original HNPCC diagnostic criteria were established by the International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer (ICG-HNPCC) and are known as the Amsterdam criteria\(^\text{[30]}\), but only 50%-70% of HNPCC families meeting these criteria have been found to have germline \textit{MSH2} or \textit{MLH1} mutations.\(^\text{[48]}\) The Amsterdam criteria were revised by the ICG-HNPCC in 1999 to include extracolonic cancers. The least stringent criteria are the Bethesda guidelines (more sensitive but less specific
than either the Amsterdam I or Amsterdam II criteria in identifying HNPCC families with pathogenic mutations), which aim to determine which patients should have MSI testing[48]. These criteria propose MSI testing for:

Individuals with cancer in families that meet the Amsterdam criteria.

Individuals with two hereditary nonpolyposis colon cancer syndrome (HNPC) related cancers, including synchronous and metachronous colorectal cancers or associated extracolonic cancers (endometrial, ovarian, gastrointestinal, hepatobiliary, or small bowel cancer or transitional cell carcinoma of the renal pelvis or ureter).

Individuals with colorectal cancer and a first-degree relative with colorectal cancer and/or HNPCC-related extracolonic cancer and/or a colorectal adenoma: one of the cancers diagnosed by age 45, and the adenoma diagnosed by age 40.

Individuals with colorectal cancer or endometrial cancer diagnosed by age 45.

Individuals with right-sided colorectal cancer with an undifferentiated pattern (solid/cribriform) on histopathology diagnosed by age 45.

Individuals with signet-ring-cell-type colorectal cancer diagnosed by age 45.

Individuals with adenomas diagnosed by age 40.

The American Gastroenterological Association recommends genetic testing for HNPCC for individuals from families meeting Amsterdam criteria, as well as for individuals with two HNPCC-related cancers (for instance, colorectal and endometrial cancer) and individuals with two HNPCC-related cancers, including extracolonic (endometrial, ovarian, gastric, hepatobiliary, or small bowel cancer or transitional cell carcinoma of the renal pelvis or ureter).

Individuals with colorectal cancer and a first-degree relative with colorectal cancer and/or HNPCC-related extracolonic cancer and/or a colorectal adenoma: one of the cancers diagnosed by age 45, and the adenoma diagnosed by age 40.

Individuals with colorectal cancer or endometrial cancer diagnosed by age 45.

Individuals with right-sided colorectal cancer with an undifferentiated pattern (solid/cribriform) on histopathology diagnosed by age 45.

Individuals with signet-ring-cell-type colorectal cancer diagnosed by age 45.

Individuals with adenomas diagnosed by age 40.

The American Gastroenterological Association recommends genetic testing for HNPCC for individuals from families meeting Amsterdam criteria, as well as for individuals with two HNPCC-related cancers (for instance, colorectal and endometrial cancer) and individuals with colorectal cancer who have a first degree relative with an HNPCC-related cancer (or colorectal adenoma) where at least one was diagnosed before age 50[49]. Ideally, testing should first be offered to a family member with colorectal or endometrial cancer[28,43,46,49]. In some individuals, genetic analysis may be offered after prescreening for MSI in an HNPCC-related tumor specimen. Such prescreening should be offered where an HNPCC-related cancer is present in two individuals related by first-degree regardless of age of onset, or individuals with early-onset CRC regardless of family history. Genetic testing is indicated if MSI is present.

The majority (90%) of mutation-positive HNPCC cases are caused by mutations in MLH1 or MSH2[3,9]. For this reason, the mutation analysis is generally performed for these two genes, MSH6 being included in the analysis more recently. Although several methods can be used to detect these mutations, direct exon-by-exon gene sequencing is considered the gold standard. The sequencing should analyze each of the protein-coding regions of the MLH1 and MSH2 genes in their entirety, with all positive results being repeated for confirmation. Once a specific mutation that has been found in a relative by previous genetic testing, a test examining only the specific portion of the gene containing the known familial mutation can be offered to all family members.

There are some benefits and limitations of genetic testing for HNPCC. Relying solely on family history can underestimate the risk of developing cancer in mutation carriers and over-estimate risk in those who do not inherit the mutation. When an individual has a personal or family history that suggests the possibility of HNPCC, an important step is to determine whether the person is interested in genetic testing. Genetic testing for HNPCC can have important benefits for members of high-risk families who choose to be tested[9]. Those who are found to carry deleterious mutations can take steps to reduce their cancer risk, especially through earlier and more intensive surveillance or consideration of prophylactic surgery. Individuals with HNPCC-related CRC can undergo surgical management designed to address the increased risk of a second cancer.

In families in whom a deleterious mutation has been found, those who are mutation-negative can be spared the need for more intensive surveillance and intervention[50]. However, these individuals remain at risk for sporadic CRC and should be encouraged to adhere to age-appropriate general population screening guidelines.

Before consenting to genetic analysis, patients should also consider the limitations of testing. Currently, genetic testing cannot detect unusual mutations responsible for HNPCC, such as those occurring in MMR genes other than MLH1 and MSH2. Therefore, a negative result in an individual who does not have a family member with a documented mutation must be interpreted cautiously. The test may also detect a variant of uncertain significance whose effect on cancer risk has not yet been established. In such situations, testing other family members for the specific variant to determine if it is associated with cancer may provide clarification of the significance[50].

**Immunohistochemical testing for MMR defects**

At the protein level, hMLH1/hMSH2 immunohistochemistry has a role in detecting MMR defects[51-53], with data suggesting that the effectiveness of immunohistochemical screening of the MMR proteins would be similar to that of the more complex strategy of microsatellite genotyping[54]. This technique can guide which gene to sequence and can help differentiating sporadic from hereditary mutations: hMLH2 loss is likely to be HNPCC, whereas hMLH1 loss could be HNPCC or sporadic CRC (MLH1 promoter methylation). MMR proteins heterodimerize to function; the hMLH2 loss almost always accompanies hMSH6 loss and when hMLH1 is lost, generally so is hPMS2[55-57]. In addition, immunohistochemistry can miss functional loss; i.e. presence of the protein with antigen positivity in the absence of function. Several antibodies have been used for these analyses, but the most widely used are hMSH2 (clone FE11, Oncogene Research), hMLH1 (clones G168 728 and G168-15, BD Pharmingen), hMSH6 (clone 44, BD Transduction Laboratories), and hPMS2 (clone A16-4, BD Pharmingen, and polyclonal C terminus, Santa Cruz Biotechnology).

MMR immunohistochemical studies are based on a complete absence of at least one MMR protein[53,55-57]. But these studies do not consider the immunostaining topographic heterogeneity[54]. Since the MMR proteins function as heterodimers, it could be advocated to validate the immunohistochemical results of hMSH2/hMSH6 and...
hMLH1/hPMS2. More studies are required to clarify the influence of this predictable tumor heterogeneity to select the appropriate sample for immunohistochemical and/or MSI analyses.

PROGNOSTIC AND THERAPEUTIC IMPLICATIONS OF MSI

The CRC microsatellite profile provides useful prognostic information\(^{8,16,30}\), showing the patients with microsatellite-unstable neoplasms have a better overall survival rate and a modified response to conventional chemotherapy\(^{4,6,7}\). MSI also helps in predicting the treatment response of CRC\(^{15,30,64,65}\), and could modify the chemotherapy protocols offered to the patients in the future\(^{66}\), but these results should be applied with caution before this predictive tool is verified\(^{67}\).

Molecular markers as predictive factors in treatment decisions have been developed in the last few years. The initial studies in sporadic CRC showed that the retention of heterozygosity at one or more 17p or 18q alleles in microsatellite-stable CRCs and mutation of the gene for the type II receptor for TGF-β1 in CRCs with high levels of microsatellite instability correlated with a favorable outcome after adjuvant chemotherapy with fluorouracil-based regimens, especially for stage III CRC\(^{63,65}\). However, most recent studies have revealed that fluorouracil-based adjuvant chemotherapy benefited patients with stage II or stage III CRC with MSS tumors or tumors exhibiting low-frequency MSI but not those with CRCs exhibiting high-frequency MSI\(^{64}\). The reasons for these responses must be related to the distinctive cell kinetics associated with MM down-regulation (significantly increased apoptosis and decreased proliferation), which can certainly contribute to tumor cell resistance to conventional chemotherapy\(^{40,41}\). The topographic heterogeneity of sporadic CRC is a key element to explain the discrepant results reported\(^{41}\). This point has not been systematically addressed yet, but a homogeneous selection of the samples from the same topography must be considered in the molecular test design\(^{40}\).

CONCLUSIONS

Many CRC show MSI, for which confirmatory analyses are warranted because of prognostic and therapeutic implications. Pathologists play a critical role in identifying microsatellite-unstable CRC, such as occur in young patients with synchronous or metachronous tumors and tumors with classic histologic features. In these cases, MSI testing and/or MMR immunohistochemistry are advisable, along with sequencing and genetic counseling if appropriate. Microsatellite analysis is an excellent functional and prognostic test, whereas MMR immunohistochemistry can guide gene sequencing but can result in false negatives (false negative in antigen positive neoplasms, especially cases with MLH1 promoter methylation). Direct exon-by-exon gene sequencing is considered the gold standard and should be used to analyze each of the protein-coding regions of the MLH1 and MSH2 genes in their entirety, although this technique will miss MLH1 gene inactivation by promoter methylation. Finally, the selection of samples for molecular tests must be carefully designed considering predictable heterogeneity, such as topographic heterogeneity, to avoid misinterpretations.

REFERENCES

1. Liu B, Parsons R, Papadopoulos N, Nicolaides NC, Lynch HT, Watson P, Jass JR, Dunlop M, Wyllie A, Peltomaki P, de la Chapelle A, Hamilton SR, Vogelstein B, Kinzler KW. Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. *Nat Med* 1996; 2: 169-174
2. Ivanovich JL, Read TE, Ciske DJ, Kodner IJ, Whelan AJ. A practical approach to familial and hereditary colorectal cancer. *Am J Med* 1999; 107: 68-77
3. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell* 1996; 87: 159-170
4. Arzimanoglou II, Gilbert F, Barber HR. Microsatellite instability in human solid tumors. *Cancer* 1998; 82: 1808-1820
5. Alexander J, Watanabe T, Wu TT, Rashid A, Li S, Hamilton SR. Histopathological identification of colon cancer with microsatellite instability. *Am J Pathol* 2001; 158: 527-535
6. Lawes D, SenGupta S, Boulos PB. The clinical importance and prognostic implications of microsatellite instability in sporadic CRC. *Eur J Surg Oncol* 2003; 29: 201-212
7. Shackney SE, Shankey TV. Common patterns of genetic evolution in human solid tumors. *Cytometry* 1997; 29: 1-27
8. Díaz-Canøo SJ, Blanes A, Wolfe HJ. PCR techniques for clonality assays. *Diagn Mol Pathol* 2001; 10: 24-33
9. Marra G, Boland CR. Hereditary nonpolyposis colorectal cancer: the syndrome, the genes, and historical perspectives. *J Natl Cancer Inst* 1995; 87: 1114-1125
10. Kim H, Jen J, Vogelstein B, Hamilton SR. Clinical and pathological characteristics of sporadic colorectal carcinomas with DNA replication errors in microsatellite sequences. *Am J Pathol* 1994; 145: 148-156
11. Mecklin JP, Sipponen P, Jarvinen HJ. Histopathology of colorectal carcinomas and adenomas in cancer family syndrome. *Dis Colon Rectum* 1986; 29: 849-853
12. Chapusot C, Martin L, Mungra N, Rageot D, Bouvier AM, Bonithon Kopp C, Ponnelle T, Faivre J, Piard F. Sporadic colorectal cancers with defective mismatch repair display a number of specific morphological characteristics: relationship between the expression of hMLH1 and hMSH2 proteins and clinicopathological features of 273 adenocarcinomas. *Histopathology* 2003; 43: 40-47
13. Greenson JK, Bonner JD, Ben-Yzak O, Cohen HJ, Miselevich I, Resnick MB, Trougouboff P, Tomsho LD, Kim E, Low M, Almog R, Rentfert G, Gruber SB. Phenotype of microsatellite unstable colorectal carcinomas: Wide-differentiated and focally mucinous tumors and the absence of dirty necrosis correlate with microsatellite instability. *Am J Surg Pathol* 2003; 27: 565-570
14. Koreth J, O’Leary JJ, O’D McGee J, Microsatellites and PCR genomic analysis. *J Pathol* 1996; 178: 239-248
15. Boland CR. Setting microsatellites free. *Nat Med* 1996; 2: 972-974
16. Brentnall TA. Microsatellite instability. Shifting concepts in tumorigenesis. *Am J Pathol* 1995; 147: 561-563
17. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science* 1993; 260: 876-879
18. Parsons R, Li CM, Longley MJ, Fang WH, Papadopoulos N, Jen J, de la Chapelle A, Kinzler KW, Vogelstein B, Modrich P. Hypermutability and mismatch repair deficiency in RER+ tumor cells. *Cell* 1993; 75: 1227-1236
19. Díaz-Canøo SJ. Designing a molecular analysis of clonality in tumours. *J Pathol* 2000; 191: 343-344
20. Sieben NL, ter Haar NT, Cornelisse CJ, Fleuren GJ, Cleton-Jansen AM. PCR artifacts in LOH and MSI analysis of
microdissected tumor cells. *Hum Pathol* 2000; 31: 1414-1419

21 **Diaz-Cano SJ.** Are PCR artifacts in microdissected samples preventable? *Hum Pathol* 2001; 32: 1415-1416

22 **Findlay I, Matthews P, Quirk P.** Multiple genetic diagnoses from single cells using multiplex PCR: reliability and allele dropout. *Prenat Diagn* 1998; 18: 1413-1421

23 **Ray PF, Handisyde AH.** Increasing the denaturation temperature during the first cycles of amplification reduces allele dropout from single cells for preimplantation genetic diagnosis. *Mid Hum Reprod* 1996; 2: 213-218

24 **Diaz-Cano SJ, Brady SP.** DNA extraction from formalin-fixed, paraffin-embedded tissues: protein digestion as a limiting step for retrieval of high-quality DNA. *Diag Mol Pathol* 1997; 6: 342-346

25 **Diaz-Cano SJ, Blanes A, Rubio J, Matilla A, Wolfe HJ.** Topographic evolution and intratumor heterogeneity by topographic compartments in muscle-invasive transitional cell carcinoma of the urinary bladder. *Lab Invest* 2000; 80: 279-289

26 **Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Grivastava S.** 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

27 **Hatch SB, Lightfoot HM Jr, Garwacki CP, Moore DT, Calvo BF, Woosley JT, Scharrota J, Funkhouser WK, Farber RA.** Microsatellite instability testing in colorectal carcinoma: choice of markers affects sensitivity of detection of mismatch repair-deficient tumors. *Clin Cancer Res* 2005; 11: 2180-2187

28 **Jarvinen HJ, Aarnio M, Mustonen H, Aktan-Collan K, Aaltonen LA, Peltomaki P, De La Chapelle A, Mecklin JP.** Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. *Gastroenterology* 2000; 118: 829-834

29 **Lynch HT, de la Chapelle A.** Genetic susceptibility to nonpolyposis colorectal cancer. *J Med Genet* 1999; 36: 801-818

30 **Vasen HF, Mecklin JP, Khan PM, Lynch HT.** The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPPC). *Dis Colon Rectum* 1991; 34: 424-425

31 **Steenwijn E, Gorunova L, Jonson T, Larsson N, Hoglund M, Mandahl N, Merten F, Mittelman F, Gisselsson D.** Structural and numerical chromosome changes in colon cancer develop through telomere-mediated anaphase bridges, not through mitotic multipolarity. *Proc Natl Acad Sci USA* 2005; 102: 15415-15420

32 **Goel A, Arnold CN, Niedzwiecki D, Chang DK, Ricciardelli L, Carethers JM, Dowell JM, Wasserman L, Compton C, Mayer RJ, Bertagnolli MM, Boland CR.** Characterization of sporadic colon cancer by patterns of genomic instability. *Cancer Res* 2003; 63: 1608-1614

33 **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

34 **Peltomaki P.** Deficient DNA mismatch repair: a common etiologic factor for colon cancer. *Hum Mol Genet* 2001; 10: 735-740

35 **Peltomaki P.** DNA mismatch repair and cancer. *Mutat Res* 2001; 488: 77-85

36 **Woerner SM, Kloor M, Mueller A, Rueschhoff J, Friedrichs N, Buettner R, Buzello M, Kiniel P, Knaebel HP, Kunzmann E, Pagenstecher C, Schackert HK, Moslein G, Vogelsang H, von Knebel Doeberitz M, Gebert JF.** Microsatellite instability of selective target genes in HNPPC-associated colon adenomas. *Oncogene* 2005; 24: 2525-2535

37 **Thibodeau SN, French AJ, Cunningham JM, Testor D, Burgart LJ, Roche PC, McDonnell SK, Schaid DJ, Vockley CW, Michels VV, Farr GH Jr, O’Connell MJ.** Microsatellite instability in colorectal cancer: different mutator phenotypes and the principal involvement of hMLH1. *Cancer Res* 1998; 58: 1713-1718

38 **Miyaki M, Iijima T, Yamaguchi T, Kadofuku T, Funata N, Mori T.** Both BRAF and KRAS mutations are rare in colorectal carcinomas from patients with hereditary nonpolyposis colorectal cancer. *Cancer Lett* 2004; 211C: 105-109

39 **Liang JT, Chang KJ, Chen JC, Lee CC, Cheng YM, Hsu HC, Chien CT, Wang SM.** Clinicopathologic and carcinogenic appraisal of DNA replication error in sporadic T3N0M0 stage colorectal cancer after curative resection. *Hepatogastroenterology* 1999; 46: 883-890

40 **Jimenez-Martin JJ, Miranda MT, Blanes A, Diaz-Cano SJ.** Expression of mlh1/msh2 in colorectal adenocarcinomas: SAGE/microarray profile and tumor phenotype. *Lab Invest* 2003; 83: 296A (Abstract).

41 **Blanes A, Jimenez-Martin JJ, Miranda MT, Diaz-Cano SJ.** Absence of the physiologic cellular kinetic balance and down-regulation of mlh1/msh2 characterize deep topographic compartments of colorectal adenocarcinomas. *Lab Invest* 2003; 83: 114A-115A (Abstract).

42 **Yamamoto H, Sawai H, Weber TK, Rodriguez-Bigas MA, Perucchini M.** Somatic frameshift mutations in DNA mismatch repair and proapoptosis genes in hereditary nonpolyposis colorectal cancer. *Cancer Res* 1998; 58: 997-1003

43 **Chapusot C, Martin L, Bovier AM, Bonithon-Kopp C, Ecarnot-Laubriet A, Ragoet D, Ponnetelle T, Laurenz Puig P, Faivre J, Piard F.** Microsatellite instability and intratumoral heterogeneity in 100 right-sided sporadic colon carcinomas. *Br J Cancer* 2002; 87: 400-404

44 **Jimenez JJ, Blanes A, Diaz-Cano SJ.** Microsatellite instability in colon cancer. *N Engl J Med* 2003; 349: 1774-1776; author reply 1774-1776

45 **Salovaara R, Lokoul A, Kristo P, Kaarinaen H, Ahtola H, Eskelinen M, Harkonen N, Julkunen R, Kangas E, Ojala S, Tulkoura J, Valkamo E, Jarvinen H, Mecklin JP, Aaltonen LA, de la Chapelle A.** Population-based molecular detection of hereditary nonpolyposis colorectal cancer. *J Clin Oncol* 2000; 18: 2193-2200

46 **Haltonen SL, Salovaara R, Kristo P, Canzian F, Hemminki A, Peltomaki P, Chadwick RB, Kaarinaen H, Eskelinen M, Jarvinen H, Mecklin JP, de la Chapelle A.** Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. *N Engl J Med* 1998; 338: 1481-1487

47 **Eng C, Hampel H, de la Chapelle A.** Genetic testing for cancer predisposition. *Annu Rev Med* 2001; 52: 371-400

48 **Syngal S, Fox EA, Eng C, Kolodner RD, Garber JE.** Sensitivity and specificity of clinical criteria for hereditary non-polyposis colorectal cancer associated mutations in MSH2 and MLH1. *J Med Genet* 2000; 37: 641-645

49 **American Gastroenterological Association medical position statement: hereditary colorectal cancer and genetic testing.** *Gastroenterology* 2001; 121: 195-197

50 **Syngal S.** Hereditary nonpolyposis colorectal cancer: a call for attention. *J Clin Oncol* 2000; 18: 2189-2192

51 **Mathiak M, Rutten A, Mangold E, Fischer HP, Ruzicka T, Friedl W, Propping P, Kruse R.** Loss of DNA mismatch repair proteins in skin tumors from patients with Muir-Torre syndrome and MSH2 or MLH1 germline mutations: establishment of immunohistochimical analysis as a screening test. *Am J Surg Pathol* 2002; 26: 338-343

52 **Southey MC, Young MA, Whitty J, Mills SD, Keilar M, Mead L, Trute L, Aitmotoki K, McLachlan SA, Debinski H, Venter DJ, Armes JE.** Molecular pathologic analysis enhances the diagnosis and management of Muir-Torre syndrome and gives insight into its underlying molecular pathogenesis. *Am J Surg Pathol* 2001; 25: 936-941

53 **Marcus VA, Madlensky L, Gryfe R, Kim H, So K, Millar A, Temple LK, Hsieh E, Hiruki T, Narod S, Bapat BV, Gallinger S, Redston M.** Immunohistochemistry for hMLH1 and hMSH2: a practical test for DNA mismatch repair-deficient tumors. *Am J Surg Pathol* 1999; 23: 1240-1255

54 **Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, Nakagawa H, Sotamaa K, Prior TW, Westman J,
Panescu J, Fix D, Lockman J, Comeras I, de la Chapelle A. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). *N Engl J Med* 2005; 352: 1851-1860

Drummond JT, Li GM, Longley MJ, Modrich P. Isolation of an hMSH2-p160 heterodimer that restores DNA mismatch repair to tumor cells. *Science* 1995; 268: 1909-1912

Lahue RS, Au KG, Modrich P. DNA mismatch correction in a defined system. *Science* 1989; 245: 160-164

Prolla TA, Pang Q, Alani E, Kolodner RD, Liskay RM. MLH1, PMS1, and MSH2 interactions during the initiation of DNA mismatch repair in yeast. *Science* 1994; 265: 1091-1093

Hawkins NJ, Ward RL. Sporadic colorectal cancers with microsatellite instability and their possible origin in hyperplastic polyps and serrated adenomas. *J Natl Cancer Inst* 2001; 93: 1307-1313

Parc YR, Halling KC, Wang L, Christensen ER, Cunningham JM, French AJ, Burgart LJ, Price-Troska TL, Roche PC, Thibodeau SN. HMSH6 alterations in patients with microsatellite instability-low colorectal cancer. *Cancer Res* 2000; 60: 2225-2231

Wahlberg SS, Schmeits J, Thomas G, Loda M, Garber J, Syngal S, Kolodner RD, Fox E. Evaluation of microsatellite instability and immunohistochemistry for the prediction of germ-line MSH2 and MLH1 mutations in hereditary nonpolyposis colon cancer families. *Cancer Res* 2002; 62: 3485-3492

Elsaleh H, Powell B, Soontrapornchai P, Joseph D, Goria F, Spyr N, Iacopetta B. p53 gene mutation, microsatellite instability and adjuvant chemotherapy: impact on survival of 388 patients with Dukes’ C colon carcinoma. *Oncology* 2000; 58: 52-59

Barrett PL, Seymour MT, Sternging SP, Georgiades I, Walker C, Birbeck K, Quirke P. DNA markers predicting benefit from adjuvant fluorouracil in patients with colon cancer: a molecular study. *Lancet* 2002; 360: 1381-1391

Ribic CM, Sargent D, Moore M, Thibodeau SN, French AJ, Goldberg RM, Hamilton SR, Laurent-Puig P, Gryfe R, Shepherd LE, Tu D, Redston M, Gallinger S. 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

Elsaleh H, Iacopetta B. Microsatellite instability is a predictive marker for survival benefit from adjuvant chemotherapy in a population-based series of stage III colorectal carcinoma. *Clin Colorectal Cancer* 2001; 1: 104-109

Liang JT, Huang KC, Lai HS, Lee PH, Cheng YM, Hsu HC, Cheng AL, Hsu CH, Yeh KH, Wang SM, Tang C, Chang KJ. 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

Chen X, Lai MD, Huang Q. Increased sensitivity of colorectal cancer cell lines with microsatellite instability to 5-fluorouracil in vitro. *Chin Med J (Engl)* 2002; 115: 1048-1052

Watanabe T, Wu TT, Catalano P, Ueki T, Satriano R, Haller DG, Benson AB 3rd, Hamilton SR. Molecular predictors of survival after adjuvant chemotherapy for colon cancer. *N Engl J Med* 2001; 344: 1196-1206

S- Editor Wang J L- Editor Lutze M E- Editor Ma WH