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Review

# Microsatellite instability in colorectal cancer

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**Summary.** Microsatellites are short tandem repeat DNA sequences of one to tetra base pairs distributed throughout the human genome, both in coding and non-coding regions. Owing to their repeated structure, microsatellites are particularly prone to replication errors that are normally repaired by the Mismatch Repair (MMR) system. MMR is a very highly conserved cellular process, involving many proteins, resulting in the identification, and subsequent repair of mismatched bases, likely to have arisen during DNA replication, genetic recombination or chemical or physical damage. Proteins within the MMR system include MLH1, PMS2, MSH2, MSH6, MLH3, MSH3, PMS1, and Exo1. Deficient MMR (dMMR) results in a strong mutator phenotype known as microsatellite instability (MSI), characterized by widespread length polymorphisms of microsatellite sequences due to DNA polymerase slippage. MSI is recognized as one of the major carcinogenetic pathways of colorectal cancer (CRC): it represents a molecular hallmark of hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome (LS); moreover it is detected in 15% of sporadic colorectal cancers, more often due to an epigenetic inactivation of MLH1. Identification of MSI CRC is important, as MSI may serve as a screening tool for detecting LS, a prognostic marker for patient outcome, and a predictive marker for response to chemotherapy and to immunotherapy. (www.actabiomedica.it)

**Key words:** microsatellite instability, colorectal cancer, mismatch repair, Lynch Syndrome, prognosis

## Background

Microsatellites are short tandem repeat DNA sequences of one to tetra base pairs distributed throughout the human genome, both in coding and non-coding regions. Owing to their repeated structure, microsatellites are particularly prone to replication errors that are normally repaired by the Mismatch Repair (MMR) system (1). MMR is a very highly conserved cellular process, involving many proteins, resulting in the identification, and subsequent repair of mismatched bases, likely to have arisen during DNA replication, genetic recombination or chemical or physical damage. Proteins within the MMR system include MLH1, PMS2, MSH2, MSH6, MLH3, MSH3, PMS1, and Exo1. These proteins form heterodimers that repair DNA damage. During normal DNA replication with proficient MMR (pMMR), small DNA mismatch errors are initially detected and bound by MSH2/MSH6 and MSH2/MSH3 heterodimers. MLH1/PMS2 heterodimers are subsequently recruited for excision and resynthesis of a new, corrected strand. However, deficient MMR (dMMR) results in a strong mutator phenotype known as microsatellite instability (MSI), characterized by widespread length polymorphisms of microsatellite sequences due to DNA polymerase slippage (2).

MSI is recognized as one of the major carcinogenetic pathways of colorectal cancer (CRC): it represents a molecular hallmark of hereditary nonpolyposis

colorectal cancer (HNPCC), also known as Lynch syndrome (LS), usually linked to a germ-line mutation in one of MMR genes; moreover it is detected in 15% of sporadic colorectal cancers, more often due to an epigenetic inactivation of MLH1 (1, 3-4).

## Clinicopathologic features of MSI CRC

Tumors with MSI are more common localized in the right colon and they are histopathologically characterized by mucinous features, tumor-infiltrating lymphocytes, poor differentiation with a medullary growth pattern, and a Crohn-like lymphocytic reaction. They are more frequent in stage II and relatively uncommon among metastatic tumors (5). Sporadic tumors typically occur in older female patients, whereas, CRC in the context of LS often occurs in younger patients (50 years of age or less). Finally, CRCs with MSI have a diploid DNA content with few losses or gains of chromosomal regions.

## Screening for MSI

Two forms of testing are commonly used in screening patients and tumors for MSI or a deficiency in MMR: polymerase chain reaction (PCR) testing for MSI and immunohistochemical staining (IHC) for altered proteins.

MSI is detected through the comparison of the length of nucleotide repeats in tumor cells and normal cells. The standard diagnostic procedure recommended by the National Cancer Institute involves analyses of tumor and normal tissues using five microsatellite markers (Bethesda panel), including two for mononucleotide repeats (BAT26 and BAT25) and three for dinucleotide repeats (D2S123, D5S346, and D17S250) (6-7). In particular, frame shift mutations in microsatellites can be identified by extraction of DNA from healthy and tumor tissue, amplification of selective microsatellites by PCR, and analysis of fragment size by capillary electrophoresis on a automated sequencer. Samples can be classified into microsatellite instability-high (MSI-H), microsatellite instability-low (MSI-L) or microsatellite stable (MSS) according the percentage of loci with MSI. In particular, the phenotype is defined as MSI-H if two or more of the five microsatellite markers show instability (or >30% of unstable markers if a larger panel is used), as MSI-L if only one of five markers shows instability and MSS if none of the markers show instability (1, 6). A new expert consensus recommends the use of a panel of 5 quasi-monomorphic mononucleotide repeats (BAT-25, BAT-26, NR21, NR24 and NR27), characterized by a constant number of nucleotide repeats and an identical size between individuals, unlike most microsatellites are polymorphic, obviating the necessity to analyze simultaneously non-tumor DNA (8). With this method, two unstable markers are sufficient to classify tumors as MSI (9).

The use of IHC to test for the MMR proteins MLH1, MSH2, MSH6 and PMS2 can be used to indicate the presence or absence of a functional MMR system, and thus, indirectly MSI, and may allow identification of the defective protein, which can then be used to direct mutation analysis to the relevant gene (10). It should be considered that MMR proteins PMS2 and MSH6 cooperate with MLH1 and MSH2 respectively and their expression closely depends on the binding to the major partner (i.e. MLH1 and MSH2). Therefore, loss of expression of MSH2 is frequently associated with loss of expression of MSH6 and this pattern is highly suggestive of MSH2 germline mutation. Similarly, loss of expression of MLH1 is frequently associated with loss of expression of PMS2 and this pattern may result either from MLH1 germline mutation or from acquired somatic hypermethylation of the MLH1 gene promoter. Germ-line mutations of MSH6 and PMS2 are generally associated with isolated loss of expression of MSH6 and PMS2 protein respectively (11). Both IHC and PCR are sensitive and specific for dMMR and MSI, and the two tests show high concordance (>95%) (12).

Recently, several groups have evaluated new methods to assess MSI using next generation sequencing (NGS) technologies from tumor and/or normal tissue pairs (13-15). NGS refers to a group of technologies which have, in common, the ability to perform and capture data from millions of sequencing reactions simultaneously, also called massively parallel sequencing (16). Hause *et al.* developed the MOSAIC meth-

od for crosssectional MSI analysis in 18 cancer types including CRC using the cancer exomes from the Cancer Genome Atlas database. This method, based on weighted-tree microsatellite instability classifier (MOSAIC) for predicting MSI status using the most informative and independent features for classifying MSI, had a high sensitivity and specificity in identifying MSIH tumors (17). However, NGS remains restricted to highly specialized laboratories and requires high quality samples from both tumor and normal tissues. These strategies are generally more expensive, as higher throughput sequencing machines and complex data processing pipelines are required.

## Clinical significance of MSI

MSI occurs in around 15% of all CRC tumors in white populations (18-19). It arises as a result of defective MMR caused by the failure of one of the four main MMR genes, MSH2, MLH1, MSH6, or PMS2. There are two different types of MMR gene failure: caused by an inherited germline mutation in one allele followed by somatic inactivation of the wild-type allele in a colonic mucosa cell (these individuals have Lynch syndrome and account for 3% to 5% of all CRCs), or failure caused by somatic inactivation of both alleles.

LS is an autosomal dominant disorder that increases the risk of developing CRC and endometrial adenocarcinoma, as well as tumors of the small intestine, stomach, ureter, renal pelvis, ovary, brain, prostate (20). Patients with LS benefit from increased surveillance; therefore, identification of patients as well as family members with this syndrome is very important. Since most (90%) CRC due to LS have MSI, MSI testing may serve as a screening tool for detecting LS.

Multiple retrospective and population-based studies have shown that patients with MSI-H CRCs have a more favorable stage-adjusted prognosis than those with MSS tumors (21-22). It has been suggested that the improved prognosis of MSI-H CRC may result from the pronounced antitumoral immune response of the host. In fact, lymphocytes infiltration, even with a Crohn's like reaction, is prominent in MSI CRCs. This is due to the lack of MMR system with the consequent accumulation of frame-shift mutations that causes the transcription and translation of peptides with altered amino acid sequences (neoantigens), that are presented by HLA class I and are recognized by cytotoxic T cells (23).

While it has been relatively well-established that the prognosis is better for patients with MSI-H CRC, whether MSI status predicts response to adjuvant chemotherapy has been more controversial. Numerous studies seem to suggest a lack of benefit of 5-FU chemotherapy in patients with MSI (24-26). On a molecular level, there is in vitro data supporting the fact that patients would need an intact MMR system to induce apoptosis of fluorouracil (FU)-modified DNA (27). Several studies supporting MSI-H as a predictive factor for improved response to irinotecan or irinotecan-based chemotherapy in CRC patients have been reported (28-29).

Recently, there has been an increased recognition of the host immune system importance in controlling tumor progression and new immunologic biomarkers have been included as a tool for the prediction of prognosis and response to therapy. MSI CRC selectively displays highly up-regulated expression of multiple immune checkpoints, including PD-1, Programmed Death-ligand 1 (PD-L1) and CTLA-4. It has been theorized that strategies involving the blockade of these immunoregulatory mechanisms might be selectively effective in this critical subset of CRC (30). Data from this study support the hypothesis that MSI tumors are more responsive to PD-1 blockade than are tumors with a proficient MMR system. Moreover, this data validates an approach for the management of a particular sub-set of tumors that is based solely on molecular status, without regard to the underlying tumor site. So, on May 2017, the Food and Drug Administration (FDA) approved pembrolizumab, a programmed death 1 (PD-1) inhibitor, for the treatment of adult and pediatric patients with unresectable or metastatic, MSI-H or dMMR solid tumors, regardless of tumor site or histology (31).

In conclusion, identification of MSI CRC is important, as MSI may serve as a screening tool for detecting LS, a prognostic marker for patient outcome, and a predictive marker for response to chemotherapy and to immunotherapy.

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