Acta Biomed 2018; Vol. 89, Supplement 9: 97-101 DOI: 10.23750/abm.v89i9-S.7960 © Mattioli 1885

Review

Microsatellite instability in colorectal cancer

Gian Luigi de'Angelis[1], Lorena Bottarelli[2], Cinzia Azzoni[2], Nicola de'Angelis[3], Gioacchino Leandro[4], Francesco Di Mario[1], Federica Gaiani[1], Francesca Negri[5]

[1] Unit of Gastroenterology and Digestive Endoscopy of Parma, University Hospital of Parma, Parma, Italy; [2] Department of Medicine and Surgery, Unit of Pathological Anatomy, University Hospital of Parma, Parma, Italy; [3] Department of Digestive, Hepatobiliary Surgery and Liver Transplantation, Henri Mondor University Hospital, Assistance Publique-Hôpitaux de Paris (AP-HP), Université Paris Est-Créteil, Créteil, France; [4] National Institute of Gastroenterology "S. De Bellis" Research Hospital, Castellana Grotte, Italy; [5]Medical Oncology Unit, University Hospital of Parma, Parma, Italy

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

Amgen Exhibit 1025
Amgen v. BMS

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.

References

1. Vilar E, Gruber SB. Microsatellite instability in colorectal cancer—the stable evidence. Nat. Rev. Clin. Oncol. 2010;7:153–62.
2. Jiricny J. The multifaceted mismatch-repair system. Nat Rev Mol Cell Biol. 2006;7(5):335–346.
3. Soreide K, Janssen EA, Soiland H, Körner H, Baak JP. Microsatellite instability in colorectal cancer. Br J Surg. 2006;93:395–406.
4. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology. 2010;138:2073–2087.
5. Jenkins MA, Hayashi S, O'Shea AM, Burgart LJ, Smyrk TC, Shimizu D, Waring PM, Ruszkiewicz AR, Pollett AF, Redston M, Barker MA, Baron JA, Casey GR, Dowty JG, Giles GG, Limburg P, Newcomb P, Young JP, Walsh MD, Thibodeau SN, Lindor NM, Lemarchand L, Gallinger S, Haile RW, Potter JD, Hopper JL, Jass JR; Colon Cancer Family Registry. Pathology features in Bethesda guidelines predict colorectal cancer microsatellite instability: a population-based study. Gastroenterology. 2007;133(1):48-56.
6. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava 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; 58:5248–57.
7. Umar A, Boland CR, Terdiman JP, Syngal S, Chapelle AD, Ruschoff J, Fishel R, Lindor NM, Burgart LJ, Hamelin R, Hamilton SR, Hiatt RA, Jass J, Lindblom A, Lynch HT, Peltomaki P, Ramsey SD, Rodriguez-Bigas MA, Vasen HF, Hawk ET, Barrett JC, Freedman AN, Srivastava S. Revised Bethesda guidelines for hereditary nonpoly-posis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004;96:261–8.
8. Suraweera N, Duval A, Reperant M, Vaury C, Furlan D, Leroy K, Seruca R, Iacopetta B, Hamelin R. Evaluation of tumor microsatellite instability using five quasimo- nomorphic mononucleotide repeats and pen-taplex PCR. Gastroenterology. 2002;123: 1804–11.
9. Goel A, Nagasaka T, Hamelin R, Boland CR. An optimized pentaplex PCR for detecting DNA mismatch repair-deficient colorectal cancers. PloS One 2010;5:e9393.
10. Lindor N, Burgart LJ, Leontovich O, Goldberg RM, Cunningham JM, Sargent DJ, Walsh-Vockley C, Petersen GM, Walsh MD, Leggett BA, Young JP, Barker MA, Jass JR, Hopper J, Gallinger S, Bapat B, Redston M, Thibodeau SN. Immunohistochemistry versus microsatellite instabilitytesting in phenotyping colorectal tumors. J. Clin. Oncol. 2002; 20 (4), 1043–1048.
11. Shia J, Tang LH, Vakiani E, Guillem JG, Stadler ZK, Soslow RA, Katabi N, Weiser MR, Paty PB, Temple LK, Nash GM, Wong WD, Offit K, Klimstra DS. Immunohistochemistry as first-line screening for detecting colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome: a 2-antibody panel may be as predictive as a 4-antibody panel. Am J Surg Pathol. 2009;33(11):1639–1645.
12. Zhang X, Li J. Era of universal testing of microsatellite instability in colorectal cancer. World J Gastrointest Oncol. 2013;5:12–19.
13. Salipante SJ, Scroggins SM, Hampel HL, Turner EH, Pritchard CC. Microsatellite instability detection by next generation sequencing. Clin. Chem. 2014:1192–1199.
14. Kim TM, Laird PW, Park PJ. The landscape of microsatellite instability in colorectal and endometrial cancer genomes. Cell. 2013;155: 858–868.
15. Cortes-Ciriano I, Lee S, Park WY, Kim TM, Park PJ. A molecular portrait of microsatellite instability across multiple cancers. Nat Commun. 2017;8:15180.
16. Frese KS, Katus HA, Meder B. Next-generation sequencing: from understanding biology to personalized medicine. Biology. 2013; 2:378–398.
17. Hause RJ, Pritchard CC, Shendure J, Salipante SJ. Classification and characterization of microsatellite instability across 18 cancer types. Nat Med. 2016;22:1342–1350.
18. Aaltonen LA, Salovaara R, Kristo P, Canzian F, Hemminki A, Peltomäki P, Chadwick RB, Kääriäinen H, Eskelinen M, Järvinen H, 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.
19. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, Nakagawa H, Sotamaa K, Prior TW, Westman J, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer) N Engl J Med. 2005;352:1851–1860.
20. Lynch HT, Snyder CL, Shaw TG, Heinen CD, Hitchins MP. Milestones of Lynch syndrome: 1895–2015. Nat Rev Cancer. 2015;15(3):181–94.
21. Samowitz WS, Curtin K, Ma KN, Schaffer D, Coleman LW, Leppert M, Slattery ML. Microsatellite instability in sporadic colon cancer is associated with an improved prognosis at the population level. Cancer Epidemiol Biomarkers Prev. 2001; 10:917–23.
22. Sinicrope FA, Rego RL, Halling KC, Foster N, Sargent DJ, La Plant B, French AJ, Laurie JA, Goldberg RM, Thibodeau SN, Witzig TE.. Prognostic impact of microsatellite instability and DNA ploidy in human colon carcinoma patients. Gastroenterology. 2006; 131:729–37.
23. Drescher KM, Sharma P, Watson P, Gatalica Z, Thibodeau SN, Lynch HT. Lymphocyte recruitment into the tumor site is altered in patients with MSI-H colon cancer. Fam cancer. 2009;8(3):231-9.
24. Ribic CM, Sargent DJ, Moore MJ, 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;349:247–57.
25. Jover R, Zapater P, Castells A, Llor X, Andreu M, Cubiella J, Balaguer F, Sempere L, Xicola RM, Bujanda L, Reñé

JM, Clofent J, Bessa X, Morillas JD, Nicolás-Pérez D, Pons E, Payá A, Alenda C; Gastrointestinal Oncology Group of the Spanish Gastroenterological Association.. The efficacy of adjuvant chemotherapy with 5-fluorouracil in colorectal cancer depends on the mismatch repair status. Eur J Cancer 2009;45365–73.
26. Kim GP, Colangelo LH, Wieand HS, Paik S, Kirsch IR, Wolmark N, Allegra CJ; National Cancer Institute. 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;25:767–72.
27. Tajima A, Hess MT, Cabrera BL, Kolodner RD, Carethers JM. The mismatch repair complex hMutS alpha recognizes 5-fluroruracil-modified DNA: Implications for chemosensitivity and resistance. Gastroenterology. 2004;127: 1678-1684.
28. Fallik D, Borrini F, Boige V, Viguier J, Jacob S, Miquel C, Sabourin JC, Ducreux M, Praz F. Microsatellite instability is a predictive factor of the tumor response to irinotecan in patients with advanced colorectal cancer. Cancer Res. 2003; 63: 5738-5744.
29. Bertagnolli MM, Niedzwiecki D, Compton CC, Hahn HP, Hall M, Damas B, Jewell SD, Mayer RJ, Goldberg RM, Saltz LB, Warren RS, Redston M. 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.
30. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz Jr LA. PD-1 blockade intumors with mismatch-Repair deficiency. N. Engl. J. Med. 2015;372 (26), 2509–2520.
31. Steven Lemery, M.D., M.H.S., Patricia Keegan, M.D., and Richard Pazdur, M.D. First FDA Approval Agnostic of Cancer Site— When a Biomarker Defines the Indication. N Engl J Med. 2017;377:1409-1412.

Correspondence:
Lorena Bottarelli,
Department of Medicine and Surgery,
Unit of Pathological Anatomy,
University Hospital of Parma, Parma, Italy;
Tel. +39 521702675;
E-mail: lorena.bottarelli@unipr.it