Oncology and Translational Medicine
DOI 10.1007/s10330-016-0188-8

February 2017, Vol. 3, No. 1, P20–P24

REVIEW ARTICLE

Implications of the mismatch repair-deficient status for the management of colorectal cancers

Qing Wei, Lin Shen (✉)

Department of Gastrointestinal Oncology, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Peking University Cancer Hospital & Institute, Beijing 100142, China

Abstract

Although the majority of colorectal cancer (CRC) cases develop through the CIN pathway, approximately 15% of cases are caused by the hypermutation known as microsatellite instability (MSI) that is a consequence of deficient (d) DNA mismatch repair (MMR). dMMR CRCs have distinct phenotypic characteristics compared with microsatellite stable (MSS) tumors. MSI CRC is associated with an earlier stage at diagnosis and improved stage-specific prognosis, although this is controversial in stage IV patients. Current evidence supports the use of adjuvant chemotherapy with fluoropyrimidine plus oxaliplatin for stage III dMMR CRC. The distinctive genomic characterization and expression profiling of dMMR CRC paves the way for tailored immunotherapies. This is supported by recent studies that highlighted the efficacy of immunotherapy in dMMR CRC. Here, we describe the molecular aspects of the MMR system and discuss the associations of MMR-deficient/MSI-H status with clinical management, especially for patients with metastatic CRC.

Received: 26 August 2016
Revised: 25 September 2016
Accepted: 25 October 2016

Key words: colorectal cancer (CRC); mismatch repair; microsatellite instability; immunotherapy

A recent study by Le DT, *et al*, highlighted remarkable responses of cancers with microsatellite instability (MSI) to anti–PD-1 inhibitors in patients who had failed conventional therapy. This finding made us re-evaluate the significance of MSI in colorectal cancer (CRC) [1]. Approximately 15% of CRCs arise from the MSI pathway that is a consequence of deficient (d) DNA mismatch repair (MMR). The frequency of MSI varies according to the tumor stage, with the highest rates among early stage cancers, with the rate decreasing with progression to locoregional and distant metastases [2]. dMMR CRCs possess many unique characteristics that make them distinguishable from other CRCs. They are notable for greater survivability, although conflicting results have been observed in stage IV patients. dMMR cases do not benefit from fluoropyrimidine-based therapy in early-stage disease as compared to proficient DNA mismatch repair (pMMR) CRCs. Nowadays, the surging interest in cancer immunotherapy, particularly checkpoint blockade, has led to a further focus on MSI tumors, which are notable for their substantial T cell infiltrates. In this review, we first summarize the clinicopathological and molecular features of the MMR system, then discuss the implications of dMMR/MSI-H status in clinical management, especially for patients with metastatic CRC.

Microsatellite instability

Microsatellites are short tandem repeat sequences that occur throughout the genome and are used as markers of dMMR. DNA polymerases are more prone to make mistakes in these regions. MSH2, MSH6, MLH1, and PMS2 are mismatch repair proteins involved in DNA repair. Following DNA replication, the MMR machinery slides along the DNA and targets mismatches for correction when it encounters them, and loss of any of the MMR repair proteins can result in frameshift mutations of microsatellites, namely, MSI.

The Cancer Genome Atla (TCGA) revealed that the MSI-H frequency in CRCs was approximately 13% [3], and details are shown in the following section. Patients with MSI due to germline mutations in one of the *MMR* genes are defined as having Lynch syndrome. Lynch syndrome accounts for approximately 3–4% of all CRCs and one-third of all cases of dMMR/MSI-associated CRC. Patients with Lynch syndrome have an elevated risk for cancers

✉ Correspondence to: Lin Shen. Email: lin100@medmail.com.cn

Table 1 MSI-H frequency in digestive system cancers

Tumor type	Frequency		Study
	n	%	
Gastric cancer	295	22	TCGA [7]
Colorectal cancer	1066	13	Hampel H, *et al* [8]
Hepatocellular carcinoma	37	16	Chiappini F, *et al* [9]
Ampullary carcinoma	144	10	Ruemmele P, *et al* [10]
Esophageal adenocarcinoma	76	7	Farris AB 3rd, *et al* [11]
Pancreatic ductal adenocarcinoma	338	0%–2%	Laghi L, *et al* [12]

of the ovaries, kidneys, bladder, stomach, small bowel, bile ducts, and brain, with the highest increase in risk for endometrial cancer (60% of women) and CRC (80% of patients) [4]. Sporadic MSI cancers develop in a background of dense promoter hypermethylation of cancer-specific genes, known as the CpG island methylator phenotype (CIMP), and they are associated with a somatic *BRAF* p.V600E mutation [5] that serves to distinguish them from Lynch syndrome cases. Less commonly, they may arise from biallelic somatic inactivation of the genes encoding an MMR component [6].

MSI-H frequency differs across cancer types. Table 1 showed the MSI-H frequency in digestive system cancers. Pancreatic ductal adenocarcinomas have a very low proportion of MSI-H cases while gastric cancer has the highest frequency.

Clinicopathological features of deficient MMR CRCs

CRC patients with dMMR tumors have distinct clinical and pathologic features that make them distinguishable from other CRCs, such as proximal colon predominance, poor differentiation, and/or mucinous histology [13]. In addition, dMMR CRC patients have a greater inflammatory state with higher serum C-reactive protein levels, dense tumor infiltrating lymphocytes (TILs), and higher platelet counts than pMMR CRC patients, as well as worse prognostic inflammatory scores based on these factors [14].

Tumors with dMMR are more common among stage II cases (almost 20%), and are relatively less common among metastatic CRCs (4%) [15]. Significantly, it has been established that dMMR CRC patients have overall better survival outcomes and are less likely to have metastases than pMMR CRC patients [16]. However, studies indicate that the better prognosis of dMMR CRC is more apparent in earlier stage tumors [17]. When a dMMR CRC metastasizes or relapses, this advantage disappears and they fare no better, if not worse, than pMMR metastatic CRC patients [18]. As we mentioned above, sporadic dMMR tumors carry somatic mutations in the *BRAF* oncogene in approximately half of cases. *BRAF* V600E shows an independent negative prognostic association with survival in microsatellite stable (MSS) CRC [19], but associations with the combination of MSI and *BRAF* have not been thoroughly investigated. Several recent studies stratified CRC patients based on MSI and *BRAF* status into three prognostic groups: MSI/*BRAF*-wild type or mutant (best prognosis), MSS/*BRAF*-wild type (intermediate prognosis), and MSS/*BRAF* mutant [20–21], although other studies have reached conflicting results [22], and no consensus exists to date on the best prognostic subgroupings.

Genomic characterization and expression profiling of dMMR CRCs

The TCGA network project revealed that CRCs could be split into three major groups—hypermutated (13%), ultramutated (3%), and those with chromosomal instability (84%) [3]. The hypermutated category has a high mutation rate of 12–40 mutations/Mb. dMMR in the hypermutated cancers results from acquired hypermethylation of the MLH1 promoter in almost all cases, leading to the silencing of expression of MLH1 and non-functioning mismatch repair, which is again in accordance with the previously discussed findings. Almost all of these tumors showed CIMP characteristics, with several other specifically tested genes also demonstrating promoter methylation. A small number of cancers show either inherited (Lynch syndrome/HNPCC) or somatic *MMR* gene mutations.

An international expert consortium [18] recently reached a consensus to describe four consensus molecular subtypes (CMS) after analysis of 18 different CRC gene expression datasets, including data from TCGA in conjunction with molecular data on mutations and (somatic copy number aberrations) SCNAs for a subset of the samples. CMS1 (MSI-immune, 14%) CRCs are hypermutated because of defective MMR with MSI and MLH1 silencing and accordingly are CIMP-high with frequent *BRAF* mutations, while having a low number of SCNAs. This equates with the previously well-characterized sporadic MSI CRC subgroup. Gene expression profiling revealed evidence of strong immune activation (immune response, PD-1 activation, NK cell, Th1 cell, and cytotoxic T cell infiltration signatures) in CMS1, consistent with pathological descriptions of prominent tumor-infiltrating $CD8^+$ cytotoxic T lymphocytes. Patients with the CMS1 subtype have a very poor survival rate after relapse. Recently, Becht *et al* [23] reported that CRC molecular subgroups and microenvironmental signatures are highly correlated. They retrospectively analyzed the composition and the functional orientation of the immune, fibroblastic, and angiogenic microenvironment of 1388 CRC tumors

from three independent cohorts using transcriptomics. The CMS1 subgroup is characterized by overexpression of genes specific to cytotoxic lymphocytes. These distinct immune orientations of the CRC molecular subtypes pave the way for tailored immunotherapies.

Treatment of MSI metastatic CRC

Predictive value of MMR status in stage II/III CRCs

Adjuvant chemotherapy in stage II tumors is controversial [24]. Limited data are currently available on the potential benefit of chemotherapy in high-risk stage II dMMR CRC. Preclinical studies have shown that dMMR tumor cells are susceptible to oxaliplatin despite displaying resistance to 5-FU [25]. The preponderance of evidence also suggests that 5-FU-based adjuvant chemotherapy is ineffective in patients with stage II dMMR tumors [26]. In the recent AGEO Study [27], the authors reported that patients with high-risk stage II dMMR CRC tended to have better outcomes with oxaliplatin-based adjuvant chemotherapy compared with surgery alone. These results need to be interpreted with caution because of the small number of patients in that subgroup. In the subgroup analysis, the disease-free survival benefit of oxaliplatin-based chemotherapy was statistically significant in multivariable analysis only in stage III cases (hazard ratio = 0.41, 95% confidence interval = 0.19 to 0.87, $P = 0.02$), consistent with the MOSAIC Study [28]. AGEO is the largest study of dMMR CRC patients, and it showed a statistically significant improvement in disease-free survival with oxaliplatin-based adjuvant chemotherapy in comparison with surgery alone in stage III patients.

MMR status and its role in the management of metastatic CRC

We have mentioned that dMMR CRCs have a greater inflammatory state, exhibited by higher serum levels of C-reactive protein and dense tumor infiltrating lymphocytes (TILs). A recent study refined these classic observations by showing that the mismatch repair–deficient tumor microenvironment strongly expresses several immune checkpoint ligands, including PD-1, PD-L1, CTLA-4, LAG-3, and IDO, which indicates that their active immune microenvironment is counterbalanced by immune inhibitory signals that resist tumor elimination [29]. Based on the results of the current and previous studies, Le DT, *et al* [1] hypothesized that dMMR/MSI CRC would have a significant clinical response to pembrolizumab (humanized anti-PD-1 antibody) treatment and a phase II clinical trial has shown strikingly positive effects in patients with MSI metastatic CRCs. As expected, whole-exome sequencing of tumor tissue revealed an average of 1,782 somatic mutations in cancers with MSI versus 73 somatic mutations in cancers without MSI. Le DT, *et al* are continuing to enroll new CRC patients in this original study. In addition, a phase III study for metastatic CRC patients has been initiated. The subjects will receive either 200 mg IV pembrolizumab (every 3 weeks for up to 35 doses) or IV mFOLFOX6/FOLFIRI-based standard therapy (every 2 weeks; NCT02563002) [30]. Multiple clinical trials studying the response of dMMR/CRC patients to pembrolizumab combined with other therapies are also underway now. For example, though limited data is available regarding the role of CTLA4 in CRC and whether anti-CTLA4 antibody therapy would be beneficial for dMMR CRC or any CRCs in general [31], a current study co-administering nivolumab (human anti-PD-1 monoclonal antibody) and ipilimumab (human anti-CTLA-4 monoclonal antibody) has been initiated for dMMR and pMMR CRC patients (NCT02060188); a treatment regimen which has been found to be more efficacious than either agent alone in melanoma trials [32–33].

Aside from pembrolizumab, other immune checkpoint inhibitors, such as the human anti-PD-L1 monoclonal antibody durvalumab, are being tested for efficacy against dMMR/MSI CRC (NCT02227667). Another dMMR CRC study is administering a combination of standard chemotherapy with the PD-L1 inhibitor, atezolizumab (800 or 1,200 mg IV every 2–3 weeks; NCT01633970). While there are no published findings on the efficacy of durvalumab or atezolizumab in CRC patients, it can be assumed that the researchers hope to find similar benefits in dMMR CRC patients as was seen in the pembrolizumab trial [34].

Testing of DNA mismatch repair and microsatellite instability

dMMR tumors can be identified by immunohistochemistry (IHC) showing lack of one or more of the MMR proteins in the tumor tissue. IHC testing does lack some sensitivity because of cases where the protein is intact but not functional. The National Cancer Institute Workshop recommended five necessary microsatellite markers to determine MSI, including two mononucleotide loci (BAT-25 and BAT-26) and three dinucleotide loci (D2S123, D5S346, and D17S250). These regions are amplified within both tumor and normal tissue via fluorescent multiplex polymerase chain reaction (PCR) and their size assessed by capillary electrophoresis [35]. Either IHC or MSI testing can be used, as both tests have a false-negative rate of 5–10% [36].

On the basis of the MSI status, CRCs can be classified into three groups, as shown in Table 2 [37]. MSI-H corresponds to dMMR, whereas MSI-L and MSS indicate pMMR. Loss of MMR protein detected by IHC has been

Table 2 Criteria for interpretation

	5 loci analyzed	> 5 loci analyzed	Interpretation
No.of markers	≥ 2	≥ 30 – 0%	MSI-H
Exhibiting instability	1	< 30 – 40%	MSI-L
Length changes	0	0	MSS or MSH-L

shown to be highly concordant with DNA-based MSI testing with a good sensitivity (> 90%) and an excellent specificity (100%) [38].

Stadler ZK *et al* [39] used the numeric mutational load of a multigene panel to identify MMR status. Thirteen percent of the patients (n = 28) exhibited MMR-D by IHC. Using the 341-gene assay, 100% of the 193 tumors with < 20 mutations were MMR-proficient. Of 31 tumors with ≥ 20 mutations, 28 (90%) were MMR-D. The three remaining tumors were easily identified as being distinct from the MMR-D tumors with > 150 mutations each. With a mutational load cutoff of ≥ 20 and < 150 for MMR-D detection, sensitivity and specificity were both 100% (95% confidence interval, 93% to 100%). A cutoff for mutational load can be identified via multigene next-generation sequencing tumor profiling, which provides a highly accurate means of screening for MMR-D using the same assay that is used for tumor genotyping.

Future directions

The promising findings from the dMMR CRC pembrolizumab clinical trials has boosted interest in immunomodulatory therapies for targeted treatment of this important CRC subtype. The next step in drug development for PD-1 inhibitors is to assess immunotherapy across tumor types. Mismatch repair testing is or will soon be integrated into standard of care algorithms. In addition, If the mechanism proposed for the efficacy of MSI-guided immunotherapy is correct, the ultimate biomarker for immunotherapeutic response is not MSI or even the mutational burden but the presence of immunogenic neoepitopes [40]. Neoantigen-based vaccinations are being studied in another clinical trial (NCT01461148) that is recruiting patients with surgically resected MSI CRC with lymph node metastases or metastasis to one or more distant organs.

It is expected that mismatch repair status and other pathogenetic biomarkers will be readily implicated in various cancer types.

Conflicts of interest

The authors indicated no potential conflicts of interest.

References

1. Le DT, Uram JN, Wang H, *et al*. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. New Engl J Med, 2015, 372: 2509–2520.
2. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol, 2005, 23: 609–618.
3. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature, 2012, 487: 330–337.
4. Lynch HT, Smyrk TC, Watson P, *et al*. Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: an updated review. Gastroenterology, 1993, 104: 1535–1549.
5. Kane MF, Loda M, Gaida GM, *et al*. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. Cancer Res, 1997, 57: 808–811.
6. Geurts-Giele WR, Leenen CH, Dubbink HJ, *et al*. Somatic aberrations of mismatch repair genes as a cause of microsatellite-unstable cancers. J Pathol, 2014, 234: 548–559.
7. Cancer Genome Atlas Research N. Comprehensive molecular characterization of gastric adenocarcinoma. Nature, 2014, 513: 202–209.
8. Hampel H, Frankel WL, Martin E, *et al*. Screening for the lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med, 2005, 352: 1851–1860.
9. Chiappini F, Gross-Goupil M, Saffroy R, *et al*. Microsatellite instability mutator phenotype in hepatocellular carcinoma in non-alcoholic and non-virally infected normal livers. Carcinogenesis, 2004, 25: 541–547.
10. Ruemmele P, Dietmaier W, Terracciano L, *et al*. Histopathologic features and microsatellite instability of cancers of the papilla of vater and their precursor lesions. Am J Surg Pathol, 2009, 33: 691–704.
11. Farris AB 3rd, Demicco EG, Le LP, *et al*. Clinicopathologic and molecular profiles of microsatellite unstable Barrett Esophagus-associated adenocarcinoma. Am J Surg Pathol, 2011, 35: 647–55.
12. Laghi L, Beghelli S, Spinelli A, *et al*. Irrelevance of microsatellite instability in the epidemiology of sporadic pancreatic ductal adenocarcinoma. PLoS One, 2012, 7: e46002.
13. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology, 2010, 138: 2073–2087.
14. Park JH, Powell AG, Roxburgh CS, *et al*. Mismatch repair status in patients with primary operable colorectal cancer: associations with the local and systemic tumour environment. Br J Cancer, 2016, 114: 562–570.
15. Roth AD, Tejpar S, Delorenzi M, *et al*. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. J Clin Oncol, 2010, 28: 466–474.
16. Malesci A, Laghi L, Bianchi P, *et al*. Reduced likelihood of metastases in patients with microsatellite-unstable colorectal cancer. Clin Cancer Res, 2007, 13: 3831–3839.
17. Roth AD, Delorenzi M, Tejpar S, *et al*. Integrated analysis of molecular and clinical prognostic factors in stage II/III colon cancer. J Natl Cancer Inst, 2012, 104: 1635–1646.
18. Guinney J, Dienstmann R, Wang X, *et al*. The consensus molecular subtypes of colorectal cancer. Nat Med, 2015, 21: 1350–1356
19. Ogino S, Shima K, Meyerhardt JA, *et al*. Predictive and prognostic roles of BRAF mutation in stage III colon cancer: results from

intergroup trial CALGB 89803. Clin Cancer Res, 2012, 18: 890–900.
20. Lochhead P, Kuchiba A, Imamura Y, *et al*. Microsatellite instability and BRAF mutation testing in colorectal cancer prognostication. J Natl Cancer Inst, 2013, 105: 1151–1156.
21. Seppälä TT, Böhm JP, Friman M, *et al*. Combination of microsatellite instability and BRAF mutation status for subtyping colorectal cancer. Br J Cancer, 2015, 112: 1966–1975.
22. French AJ, Sargent DJ, Burgart LJ, *et al*. Prognostic significance of defective mismatch repair and BRAF V600E in patients with colon cancer. Clin Cancer Res, 2008, 14: 3408–3415.
23. Becht E, de Reyniès A, Giraldo NA, *et al*. Immune and Stromal Classification of Colorectal Cancer Is Associated with Molecular Subtypes and Relevant for Precision Immunotherapy. Clin Cancer Res, 2016, 22: 4057–4066.
24. Tournigand C, Andre T, Bonnetain F, *et al*. Adjuvant therapy with fluorouracil and oxaliplatin in stage II and elderly patients (between ages 70 and 75 years) with colon cancer: subgroup analyses of the multicenter international study of oxaliplatin, fluorouracil, and leucovorin in the adjuvant treatment of colon cancer trial. J Clin Oncol, 2012, 30: 3353–3360.
25. Fink D, Nebel S, Aebi S, *et al*. The role of DNA mismatch repair in platinum drug resistance. Cancer Res, 1996, 56: 4881–4886.
26. Vilar E, Gruber SB. Microsatellite instability in colorectal cancer-the stable evidence. Nat Rev Clin Oncol, 2010, 7: 153–162.
27. Tougeron D, Mouillet G, Trouilloud I, *et al*. Efficacy of adjuvant chemotherapy in colon cancer with microsatellite instability: a large multicenter AGEO study. J National Cancer Institute, 2016, 108: 6144–6152.
28. André T, de Gramont A, Vernerey D, *et al.* Adjuvant fluorouracil, leucovorin, and oxaliplatin in stage II to III colon cancer: updated 10-year survival and outcomes according to BRAF mutation and mismatch repair status of the MOSAIC study. J Clin Oncol, 2015, 33: 4176–4187.
29. Llosa NJ, Cruise M, Tam A, *et al.* The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. Cancer Discov, 2015, 5: 43–51.
30. Diaz LA, Le DT, Yoshino T, *et al.* KEYNOTE-177: First-line, open-label, randomized, phase III study of pembrolizumab (MK-3475) versus investigator-choice chemotherapy for mismatch repair deficient or microsatellite instabilityhigh metastatic colorectal carcinoma. J Clin Oncol, 2016, 34: 4s.
31. Chung KY, Gore I, Fong L, *et al.* Phase II study of the anti-cytotoxic T-lymphocyte-associated antigen 4 monoclonal antibody, tremelimumab, in patients with refractory metastatic colorectal cancer. J Clin Oncol, 2010, 28: 3485–3490.
32. Wolchok JD, Kluger H, Callahan MK, *et al*. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med, 2013, 369: 122–133.
33. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med, 2015, 373: 23–34.
34. Quiroga D, Lyerly HK, Morse MA. Deficient Mismatch Repair and the Role of Immunotherapy in Metastatic Colorectal Cancer. Curr Treat Options Oncol, 2016, 17: 41.
35. Buhard O, Cattaneo F, Wong YF, *et al*. Multipopulation analysis of polymorphisms in five mononucleotide repeats used to determine the microsatellite instability status of human tumors. J Clin Oncol, 2006, 24: 241–251.
36. Umar A, Boland CR, Terdiman JP, *et al*. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst, 2004, 96: 261–268.
37. Boland CR, Thibodeau SN, Hamilton SR, *et al*. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res, 1998, 58: 5248–5257.
38. Lindor NM, Burgart LJ, Leontovich O, *et al*. Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors. J Clin Oncol, 2002, 20: 1043–1048.
39. Stadler ZK, Battaglin F, Middha S, *et al*. Reliable detection of mismatch repair deficiency in colorectal cancers using mutational load in next-generation sequencing panels. J Clin Oncol, 2016, 34: 2141–2147.
40. Dudley JC, Lin MT, Le DT, *et al*. Microsatellite Instability as a Biomarker for PD-1 Blockade. Clin Cancer Res, 2016, 22: 813–820.

DOI 10.1007/s10330-016-0188-8
Cite this article as: Wei Q, Shen L. Implications of the mismatch repair-deficient status for the management of colorectal cancers. Oncol Transl Med, 2017, 3: 20–24.