Molecular and prognostic heterogeneity of microsatellite-unstable colorectal cancer

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Abstract
Colorectal cancers (CRCs) with a high level of microsatellite instability (MSI-H) are clinicopathologically distinct tumors characterized by predominance in females, proximal colonic localization, poor differentiation, mucinous histology, tumor-infiltrating lymphocytes, a Crohn’s-like lymphoid reaction and a favorable prognosis. In terms of their molecular features, MSI-H CRCs are heterogeneous tumors associated with various genetic and epigenetic alterations, including DNA mismatch repair deficiency, target microsatellite mutations, BRAF mutations, a CpG island methylator phenotype-high (CIMP-H) status, and a low level of genomic hypomethylation. The molecular heterogeneity of MSI-H CRCs also depends on ethnic differences; for example, in Eastern Asian countries, relatively low frequencies of CIMP-H and BRAF mutations have been observed in MSI-H CRCs compared to Western countries. Although the prognostic features of MSI-H CRCs include a favorable survival of patients and low benefit of adjuvant chemotherapy, there may be prognostic differences based on the molecular heterogeneity of MSI-H CRCs. Here, we have reviewed and discussed the molecular and prognostic features of MSI-H CRCs, as well as several putative prognostic or predictive molecular markers, including HSP110 expression, beta2-microglobulin mutations, myosin 1a expression, CDX2/CK20 expression, SMAD4 expression, CIMP status and LINE-1 methylation levels.

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Key words: Colorectal cancer; Microsatellite instability; DNA mismatch repair; DNA methylation; CpG islands; Prognosis; Adjuvant chemotherapy

Core tip: A high level of microsatellite instability (MSI-H) is a known molecular indicator of a favorable prognosis and low benefit of 5-fluorouracil-based adjuvant chemotherapy in patients with colorectal cancer (CRC). However, MSI-H CRCs are molecularly heterogeneous tumors, which are characterized by DNA mismatch repair deficiency and various genetic and epigenetic alterations. Therefore, we hypothesized that MSI-H CRCs can be divided into prognostic subgroups based on the molecular heterogeneity. This article provides an up-to-date review concerning the underlying molecular features of MSI-H CRCs and potential prognostic or predictive molecular markers for MSI-H CRCs.

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INTRODUCTION
Microsatellite instability (MSI) is a unique molecular alteration induced by deficiencies in the DNA mismatch repair (MMR) system and is characterized by unstable (length-changeable) microsatellites, a type of simple DNA sequence repeat. The MSI phenotype has been regarded as one of the main molecular subtypes of colorectal cancers (CRCs) and accounts for 12%-20% and 6%-13% of CRCs in Western and Eastern countries, respectively. Hereditary CRCs with a high level of MSI (MSI-H) constitute approximately 3%-5% of all CRCs and arise exclusively in patients with Lynch syndrome. Lynch syndrome was formerly termed hereditary non-polyposis colorectal cancer and is caused by a germline mutation in at least one of the MMR genes (MLH1, MSH2, PMS2, and MSH6), frequently resulting in the development of early-onset malignancies, including CRC and endometrial cancer. Sporadic MSI-H CRCs account for approximately 3%-15% of all CRCs and develop mainly as a result of inactivation of the MLH1 gene via promoter CpG island hypermethylation.

MSI status in CRCs can be determined by DNA testing using microsatellite markers, and five microsatellite markers recommended by the National Cancer Institute (NCI) workshop have been officially used for MSI analysis: BAT25, BAT26, D2S123, D5S346 and D17S250. In DNA analysis using these NCI markers, instability observed in two or more of the five markers corresponds to MSI-H. MSI-H can be interpreted as the presence of MSI. In contrast, a low level of MSI (MSI-L), which is assigned when only one unstable marker is detected, is not regarded as a true MSI-positive status. Microsatellite-stable (MSS) status can be assigned when all of the markers show stability. Immunohistochemistry (IHC) for MMR proteins can be applied as a screening test or a supportive test for MSI analysis.

MSI-H CRC is known to have distinct clinicopathological and molecular features, including preferential localization in the proximal colon, a less advanced cancer stage, extracellular mucin production, medullary carcinoma and poorly differentiated carcinoma, tumor-infiltrating lymphocytes, a Crohn's-like lymphoid reaction, and a BRAF V600E mutation. In addition, and more importantly, it has been consistently reported that MSI-H CRC is associated with favorable survival and chemotherapy resistance. In a considerable number of previous studies, patients with MSI-H CRC demonstrated a significantly better survival than MSS/MSI-L CRC patients, whereas the beneficial effect of 5-fluorouracil (5-FU)-based adjuvant chemotherapy in patients with MSI-H CRC has been controversial. These prognostic features of MSI-H CRCs have been increasingly reported, and MSI is currently regarded as a molecular marker indicating favorable prognosis for CRCs. However, because MSI-H CRCs are characterized by various underlying molecular changes, including a defective MMR (dMMR) system and genetic and epigenetic alterations, it is likely that molecular factors for the stratification of patient prognosis and prediction of chemotherapy response in MSI-H CRCs could be identified. On the basis of this hypothesis, we reviewed the literature and provide information about several putative prognostic molecular factors for MSI-H CRCs.

MOLECULAR HETEROGENEITY OF MSI-H CRC

DNA MMR deficiency
Germline mutation or sporadic methylation of MMR genes: As described above, MSI is caused by the inactivation of at least one of the MMR genes. The inactivation of MMR genes can be induced by a germline mutation or by promoter CpG island hypermethylation. Germline mutation of MMR genes, including MLH1, MSH2, PMS2, or MSH6, represents a major cause of hereditary MSI-H CRCs in Lynch syndrome. Among these MMR genes, germline mutations in the MLH1 or MSH2 genes account for the majority of Lynch syndrome CRCs. Promoter methylation of MMR genes is a major cause of sporadic MSI-H CRCs and exclusively involves the MLH1 gene. MLH1 promoter methylation is closely associated with the CpG island methylator phenotype (CIMP) in sporadic CRCs and has been used as one of the major molecular markers for CIMP determination in CRCs. It is also generally expected that all of the MLH1-methylated MSI-H CRCs are CIMP-high (CIMP-H) tumors, although discordance between MLH1 methylation and CIMP status can be observed in a small subset of MSI-H CRCs. A detailed review and discussion concerning the molecular basis and prognostic implication of CIMP in MSI-H CRCs will be presented in the following sections.

Constitutional (germline) epimutation of MMR genes: Promoter methylation of MSH2 owing to germline deletion of 3' exons in the EPCAM gene (also known as the TACSTD1 gene), which is located in the region immediately upstream of MSH2, was recently identified as a cause of MSI-H CRC in Lynch syndrome. This molecular alteration is associated with a small subset of patients with Lynch syndrome and is also called the MSH2 “epimutation” because of its unique feature of heritable constitutional epigenetic change. In addition, constitutional epimutation of the MLH1 gene has also been found in a few patients with Lynch syndrome. Although it has been reported that an association between MLH1 epimutation and a family history of CRC is not evident, the clinical and pathological significance of epimutations in MMR genes in hereditary MSI-H CRCs.

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remains unclear. According to our data, MLH1 methylation-positive/CIMP-negative tumors account for 7.3% of MSI-H CRCs\(^{[29]}\), and these cases were associated with an early age of onset and favorable survival. It cannot be excluded that MLH1 epimutation-associated MSI-H CRCs may be included in these MLH1 methylation-positive/CIMP-negative MSI-H CRCs, and the predominance of young patients can imply the presence of MLH1 epimutation carriers. Thus, additional studies should be performed to investigate the detailed epidemiology and clinical implications of MMR gene epimutations in MSI-H CRC.

Expression profile of MMR proteins: Normal DNA MMR function is executed by MMR protein complexes composed of heterodimers of MutL homologues (the MLH or PMS series) or MutS homologues (the MSH series). The major role of the human DNA MMR system is performed by the MutS\(\alpha\) and MutL\(\alpha\) complexes. The MutS\(\alpha\) complex comprises a MSH2-MSH6 heterodimer, whereas the dimer of MLH1 and PMS2 forms the MutL\(\alpha\) complex\(^{[21,35]}\). These complexes are essential components of the human DNA MMR machinery, and defects in any one of these four MMR proteins lead to a dysfunctional MMR system and ultimately result in MSI. Therefore, loss of expression of MMR proteins can serve as a molecular hallmark of a dMMR system and the MSI-H status in tumors. IHC for MMR proteins is a simple and valuable tool for the detection of dMMR CRC and can be helpful for investigating the underlying molecular alteration and hereditary/sporadic status of MSI-H CRCs. The immunohistochemical profile of four MMR proteins in MSI-H CRCs can be summarized as four expression phenotypes: MLH1-negative/PMS2-negative, PMS2-negative only, MSH2-negative/MSH6-negative, and MSH6-negative only\(^{[8,36]}\). These four phenotypes most likely represent inactivation of MLH1, PMS2, MSH2, and MSH6, respectively. The majority of MSI-H CRCs are induced by inactivation of MLH1 or MSH2, whereas inactivation of PMS2 or MSH6 causes only a minor portion of MSI-H CRCs. Interestingly, an inactivating mutation or methylation of MLH1 is accompanied by PMS2 loss, and inactivation of MSH2 is combined with the loss of MSH6 expression due to their heterodimer structures. However, inactivation (mainly through germline mutations) of PMS2 or MSH6 does not accompany a loss of MLH1 or MSH2 expression, respectively\(^{[8,36]}\). Although IHC for MLH1 and MSH2 has generally been used for the screening of MSI status in CRCs, the inclusion of screening for PMS2 and MSH6 would compensate for the equivocal results of MLH1 or MSH2 IHC and aid in the detection of rare MSI-H CRCs with germline mutations of PMS2 or MSH6.

Genetic alterations

Microsatellite mutations: A recent study elucidating the molecular landscape of CRC by The Cancer Genome Atlas Project reported that the hypermutated phenotype mainly overlaps with MSI-H status in CRCs\(^{[37]}\). This finding is not surprising because MSI-H status tumors are highly vulnerable to insertions or deletions in microsatellite sequences, as described above. In CRCs with MSI, many types of genetic mutations can occur, but the majority of mutations that develop under MSI-H status are frameshift mutations because the instability of microsatellite sequences in coding regions can alter entire reading frames adjacent to the insertion or deletion point. Previous investigations have reported frameshift mutations of various genes caused by the instability of microsatellites in MSI-H CRCs. The target genes for microsatellite mutations in MSI-H CRCs include TGFBR2, BAX, ACVR2, IGF2R, BLM, MSH3, MSH6, E2F4, PTEN, AIM2, CASPASE3, MBD4, TCF4, STK11, RAD50, CHK1, AXIN2, WISP3, B2M, MYO1A and CDX2\(^{[38-54]}\). These genes are known to be associated with important biological functions such as signal transduction, apoptosis regulation, cell cycle regulation, cell proliferation, cell differentiation, and DNA MMR; therefore, loss-of-function mutations in these genes can critically contribute to tumorigenesis. However, although the biological and clinical implications of mutations in these genes in CRCs have been explored, their potential use as prognostic markers or therapeutic targets has not been established. For instance, although mutations in TGFBR2 and BAX, which are representative target tumor suppressor genes for microsatellite mutations in MSI-H CRCs, have been previously analyzed to determine their prognostic implications, conflicting results have been reported. A few groups have reported that TGFBR2 and BAX mutations were associated with a favorable prognosis in MSI-H CRCs\(^{[35,56]}\), whereas other investigators did not find a prognostic significance of these mutations in MSI-H CRCs\(^{[57,58]}\). Interestingly, microsatellite alterations in intronic regions could induce mutations in genes such as HSP110 and MRE11 in MSI-H CRCs\(^{[59]}\). In the recent study by Dorard et al\(^{[59]}\), deletions of the Tp+ mononucleotide repeat located in intron 8 of the HSP110 gene were shown to lead to exon 9 skipping and the production of truncated mutant proteins of HSP110. The HSP110 mutation as a potential prognostic and predictive marker in MSI CRC-H will be discussed briefly in the “Putative prognostic or predictive molecular markers for MSI-H CRC” section.

BRAF/KRAS mutations: BRAF is a member of the RAS family of genes and is a downstream effector of the KRAS gene, whereas KRAS is a member of the RAS family of genes and is a downstream effector of the EGFR gene in the Ras-Raf-MEK-ERK signaling pathway. The Ras-Raf-MEK-ERK signaling pathway is commonly involved in cell cycle progression and cell proliferation, and thus, activating mutations of key component genes in this pathway, including mutations in the BRAF or KRAS gene, can bring about uncontrolled cell growth and increased cell survival and may play an important role in tumorigenesis. BRAF and KRAS mutations are mutually exclusive in cancers, and the majority of mutations of BRAF and KRAS in human tumors are
Epigenetic alterations

CIMP: CIMP represents a distinct subset of CRCs that show extensive promoter CpG island methylation and are characterized by transcriptional repression of many tumor suppressor genes as a result of promoter methylation. The CIMP status of CRCs can be classified into three subtypes: CIMP-H, CIMP-low (CIMP-L), and CIMP-zero (CIMP-0). Among these subtypes, CIMP-H is generally regarded as the true CIMP-positive status. Previous investigations have revealed that CIMP-H is highly associated with sporadic MSI-H owing to the high frequency of MLH1 promoter methylation in CIMP-H CRCs. According to data from Western countries, CIMP-H CRCs account for 7%-29% of all CRCs, and 56%-82% of MSI-H CRCs have the CIMP-H subtype (Table 1). In our previous study investigating differential clinicopathological features of MSI-H CRCs depending on CIMP status, we found that the CIMP-H subtype was significantly associated with older age, frequent BRAF V600E mutations, poor differentiation, medullary carcinoma components, and signet ring cell carcinoma components in MSI-H CRCs. The effect of CIMP status on MSI-H CRC prognosis was discussed in the “Putative prognostic or predictive molecular markers for MSI CRC” section.

Genome-wide DNA methylation: The genomic methylation levels of specific tissues can be estimated by measuring the methylation levels of repetitive DNA elements, such as long interspersed nucleotide element-1 (LINE-1) and Alu, because these repetitive elements are globally distributed and occupy considerable portions of the human genome. Of these repetitive elements, the methylation level of LINE-1 has been generally used as a reliable surrogate marker for the global DNA methylation level. In particular, LINE-1 hypomethylation has been regarded as one of the molecular characteristics that distinguishes CRC tumors from normal tissue. Interestingly, several previous investigations have reported that LINE-1 hypomethylation is inversely correlated with MSI-H status in CRCs. In a study by Ogino et al., a relatively high level of LINE-1 methylation was significantly associated with both MSI-H and CIMP-H statuses in CRCs. Notably, in this study, a correlation between the LINE-1 methylation level and MSI status was significant regardless of CIMP status; furthermore, a low LINE-1 methylation level was associated with 18q loss of heterozygosity (LOH) in CRCs. These findings suggest that genomic hypomethylation may be a characteristic phenomenon of the chromosomal instability (CIN) pathway, rather than the MSI pathway, in colorectal carcinogenesis. However, the mechanistic correlation between MSI status and resistance to genomic hypomethylation in CRCs should be further evaluated. The prognostic value of the LINE-1 methylation level in MSI-H CRCs will be described below, in the “Putative prognostic or predictive molecular markers for MSI-H CRC” section.

Molecular heterogeneity among ethnic groups

As mentioned above, MSI-H CRCs constitute approximately 15% of all CRCs in Western countries. However, according to previous studies reported by our

Table 1 Frequency of CpG island methylator phenotype-high in colorectal cancers: A review of the literature

| Ref. | Country     | CIMP-H CRCs/tested CRCs n (%) |
|------|-------------|------------------------------|
|      |             | In all CRCs | In MSI-H CRCs |
| Western countries |  |  |  |
| Samowitz et al., 2005 United States | 250/859 (29.1) | 64/78 (82.1) |
| Weisenberger et al., 2006 United States | 33/187 (17.6) | NA |
| Samowitz et al., 2006 United States | 313/1271 (24.6) | 105/170 (61.8) |
| Barault et al., 2008 France | 95/578 (16.4) | 58/80 (72.5) |
| Ogino et al., 2009 United States | 123/631 (19.5) | 86/118 (72.9) |
| Dahlin et al., 2010 Sweden | 46/411 (11.2) | 34/61 (55.7) |
| Zlobec et al., 2011 Switzerland | 22/314 (7) | NA |
| Lochhead et al., 2013 United States | 285/1173 (17.5) | 140/184 (76.1) |
| Eastern countries |  |  |  |
| Koizuma et al., 2004 Japan | NA | 16/28 (57.1) |
| Nagasaka et al., 2008 Japan and Germany | NA | 15/36 (41.7) |
| Kim et al., 2009 South Korea | 29/271 (10.7) | 8/33 (24.2) |
| Min et al., 2011 South Korea | 34/245 (13.9) | NA |
| Bae et al., 2013 South Korea | 47/734 (6.4) | 18/65 (27.7) |
| Kim et al., 2013 South Korea | 285/1173 (17.5) | 140/184 (76.1) |

1MLH1-methylated colorectal cancers (CRCs) instead of CpG island methylator phenotype-high (CIMP-H) CRCs; 2Sporadic microsatellite instability-high (MSI-H) CRCs instead of CIMP-H CRCs; NA: Not applicable.

hot spot mutations in codon 600 (V600E) and codons 12 or 13, respectively. According to previous studies, BRAF mutations were found in 5%-15% of overall CRCs, whereas 32%-40% of CRCs have KRAS mutations. However, it has been revealed that BRAF V600E mutations are highly associated with MSI-H CRCs, although the incidence of KRAS mutations is inversely correlated with MSI-H status in CRCs. The frequencies of BRAF and KRAS mutations in MSI-H CRCs have been reported to be 16%-52% and 12%-20%, respectively, in Western countries. Notably, because BRAF V600E mutations have been found exclusively in sporadic tumors among MSI-H CRCs, it has been suggested that detection of BRAF mutations in CRCs may be a useful supportive tool for distinguishing sporadic CRCs from Lynch syndrome CRCs. In fact, it is thought that this observed correlation between BRAF mutations and sporadic MSI-H CRCs is mostly based on the more significant association between BRAF mutations and CIMP-H status in CRCs. Regarding the implications for prognosis, several previous studies have reported that BRAF mutations indicate poor survival in patients with CRC. However, it has been suggested that the contribution of BRAF mutations to an adverse prognosis is significant for MSI-L/MSS CRCs but not MSI-H CRCs. Therefore, the clinical and prognostic significance of BRAF mutations in MSI-H CRCs should be carefully explored in larger samples.
In all CRCs | In MSI-H CRCs
--- | ---
Western countries
Samowitz et al.\(^1\) \(2005\) | United States | 86/859 (10) | 43/78 (55.1)
Weisenberger et al.\(^2\) \(2006\) | United States | 26/187 (13.9) | NA
Samowitz et al.\(^2\) \(2006\) | United States | 123/1271 (9.7) | 67/170 (39.4)
Goel et al.\(^3\) \(2007\) | United States | 26/126 (20.6) | 17/24 (70.8)
Maestro et al.\(^4\) \(2007\) | Spain | 12/324 (3.7) | 9/49 (18.4)
Barault et al.\(^5\) \(2008\) | France | 76/578 (13.1) | 51/80 (63.8)
French et al.\(^6\) \(2008\) | United States | 77/490 (15.7) | 35/58 (60.3)
Ogino et al.\(^7\) \(2009\) | United States | 107/631 (16.9) | 51/118 (44.9)
Richman et al.\(^8\) \(2009\) | United Kingdom | 56/710 (7.9) | NA
Kumar et al.\(^9\) \(2009\) | United States (African) | 7/98 (7.1) | 7/30 (23.3)
Vilkin et al.\(^10\) \(2009\) | Israel | 24/128 (18.8) | 6/13 (46.2)
Roth et al.\(^11\) \(2010\) | Europe | 203/1309 (7.9) | 45/188 (23.9)
Dahlén et al.\(^12\) \(2010\) | Sweden | 55/411 (13.4) | 34/61 (55.7)
Zehetleitner et al.\(^13\) \(2011\) | Austria | 42/314 (13.4) | NA
Tiet et al.\(^14\) \(2011\) | Australia | 52/525 (9.9) | 24/75 (32)
Yamauchi et al.\(^15\) \(2012\) | United States | 185/1276 (14.3) | NA
Kalady et al.\(^16\) \(2012\) | United States | 56/475 (11.8) | 29/76 (38.2)
Tian et al.\(^17\) \(2013\) | The Netherlands and Spain | 42/381 (11) | NA
Lochhead et al.\(^18\) \(2013\) | United States | 182/1253 (14.5) | 101/195 (52.3)
Eastern countries
Koinuma et al.\(^19\) \(2006\) | Japan | 16/14 (11.4) | 12/26 (42.9)
Nagasaka et al.\(^20\) \(2004\) | Japan and Australia | 21/234 (9) | 16/35 (45.7)
Chang et al.\(^21\) \(2006\) | Taiwan | 9/213 (4.2) | 7/19 (36.8)
Nagasaka et al.\(^22\) \(2008\) | Japan and Germany | 20/243 (8.2) | 10/36 (27.8)
Kim et al.\(^23\) \(2009\) | South Korea | 13/271 (4.8) | 3/33 (9.1)
Yagi et al.\(^24\) \(2010\) | Japan | 13/149 (8.7) | NA
Shen et al.\(^25\) \(2011\) | China | 2/118 (17) | NA
Liu et al.\(^26\) \(2011\) | Taiwan | 12/314 (3.8) | NA
Yokota et al.\(^27\) \(2011\) | Japan | 15/229 (6.6) | NA
Aoyagi et al.\(^28\) \(2011\) | Japan | 1/134 (0.7) | NA
Kwon et al.\(^29\) \(2011\) | South Korea | 4/92 (4.3) | NA
Min et al.\(^30\) \(2011\) | South Korea | 11/249 (4.5) | 6/49 (12.2)
Fishe et al.\(^31\) \(2012\) | Taiwan | 2/382 (1.3) | NA
Nakanishi et al.\(^32\) \(2012\) | Japan | 17/254 (6.7) | 11/31 (35.5)
Bae et al.\(^33\) \(2013\) | South Korea | 39/728 (5.4) | 4/65 (6.2)
Kim et al.\(^34\) \(2013\) | South Korea | 26/219 (11.9)

CRC: Colorectal cancer; MSI: Microsatellite instability; NA: Not applicable.

group and others, a relatively low frequency of MSI-H (5.5%-9.4%) has been consistently observed in Korean patients with CRC, regardless of the institutions at which the study samples were collected.\(^{6,25,87,94-97}\) Furthermore, the frequencies of CIMP-H and BR-CAF V600E mutations in CRCs are lower in Koreans (6.4%-13.9% and 4.3%-5.4%, respectively) than in Western populations.\(^{97-100}\) We hypothesized that the low frequency of MSI-H CRCs in Korea is mainly based on the low prevalence of CIMP-H CRCs and that there are ethnic differences in the major molecular alterations associated with CRCs. Therefore, we performed a literature review to assess the frequencies of CIMP-H and BR-CAF mutations in CRCs, and the results are summarized in Tables 1 and 2, respectively. CIMP-H CRCs account for 7%-29.1% of all CRCs and 55.7%-82.1% of MSI-H CRCs in Western countries (Table 1)\(^{11,13,22,24,63,84-86}\), whereas CIMP-H CRCs constitute 6.4%-13.9% of all CRCs and 24.2%-57.1% of MSI-H CRCs in Eastern Asian countries (Table 1)\(^{11,13,99,101,102}\).

In Western countries, the BR-CAF V600E mutations were present in 3.7%-20.6% of all CRCs and in 18.4%-70.8% of MSI-H CRCs (Table 2)\(^{4,13,22,24,63,79,84-86,103-112}\). These proportions were lower, at 0.7%-11.4% of all CRCs and 6.2%-45.7% of MSI-H CRCs, in Eastern Asian countries (Table 2)\(^{11,13,99,101,112}\). These findings suggest that the Eastern Asian ethnicity is associated with a relatively low prevalence of CIMP-H and BR-CAF mutations in CRCs; consequently, these epidemiologic features may also result in a low frequency of MSI-H status in CRCs because it is thought that the majority of sporadic MSI-H CRCs are derived from CIMP-H CRCs. Thus, the low incidence of CIMP-H and BR-CAF mutations in Eastern Asian patients with CRC may be due to genetic or environmental differences between Eastern and Western ethnic groups. However, the detailed epidemiology and causal factors of the molecular heterogeneity of CRCs between ethnic groups should be elucidated in further investigations.

### PROGNOSTIC HETEROGENEITY OF MSI-H CRC

#### Prognostic features and chemotherapy responses of MSI-H CRC

Although several previous investigations have failed to identify prognostic significance of MSI status in CRCs,\(^{118,122}\), it has been consistently reported that patients with MSI-H CRC show a better survival than MSI-L/ MSS CRC patients.\(^{113,114}\) However, there has been controversy regarding the predictive value of MSI status for the response to adjuvant chemotherapy in patients with CRC.\(^{112-114}\) Regardless of the controversy, it is generally agreed that there are fewer or no beneficial effects of adjuvant chemotherapy, especially 5-FU-based chemotherapy, for patients with MSI-H CRC compared to patients with MSI-L/MSS CRC.\(^{17,19,125-127}\) According to previous in vitro experiments, the preservation of MMR function in cancer cells is likely important for inducing the apoptotic effect of 5-FU,\(^{128-131}\), and this finding could explain the molecular basis of resistance to 5-FU-based chemotherapy in MSI-H CRCs. In contrast to the tendencies towards a poor response to 5-FU, and although the findings remain controversial,\(^{112}\) several studies supporting MSI-H as a predictive factor for improved response to irinotecan or irinotecan-based chemotherapy in CRC patients have been reported.\(^{113,134}\) In previous experiments, it has also been suggested that mutations of MREI1 and/or RAD50 found in MSI-H CRC cells could account for increased sensitivity to irinotecan.\(^{113,134}\) Concerning the response to the leucovorin/5-FU/oxaliplatin (FOLFOX) regimen in CRC patients, a few investigations have suggested that MSI is associated with improved...
survival in CRC patients who are treated with adjuvant FOLFOX\textsuperscript{[59]}, whereas other studies have reported that MSI status was not significantly associated with a survival benefit of CRC patients after adjuvant FOLFOX treatment\textsuperscript{[138-141]}. Collectively, in patients with CRC, although MSI-H can indicate a better prognosis than MSI-L/MSS status, whether there is an association between MSI status and response to adjuvant chemotherapy remains controversial. Thus, further investigation is needed to confirm the predictive value of MSI status regarding responses to various chemotherapy regimens for CRCs.

**Putative prognostic or predictive molecular markers for MSI-H CRC**

Although MSI is known to be a molecular factor indicating a favorable prognosis in CRCs, we hypothesized that prognostic heterogeneity based on molecular heterogeneity might be present in MSI-H CRCs. Therefore, we expected that molecular markers stratifying patient prognosis or predicting chemotherapy response among MSI-H CRCs could be identified (Figure 1). By performing a review of the literature, potential prognostic or predictive molecular markers in MSI-H CRC were identified and are summarized in Table 3. These markers are introduced and discussed in following sections.

**HSP110:** Dorard et al\textsuperscript{[59]} recently reported that the expression level of mutant HSP110 (heat shock protein 110 kDa) is significantly associated with prognosis and chemotherapy response in patients with MSI-H CRCs. According to this study, the T\textsuperscript{r} mononucleotide repeat located within intron 8 of *HSP110* is vulnerable to deletions under the dMMR condition in CRCs, and these deletions can lead to exon 9 skipping and the generation of a truncated mutant HSP110 (HSP110\textsuperscript{ΔE9}). MSI-H CRC patients with a high mRNA expression level of HSP110\textsuperscript{ΔE9} survived longer, and this improved survival was maintained in both stage III and adjuvant chemotherapy-treated subgroups\textsuperscript{[59]}. In our recent study, we evaluated the expression status of wild-type HSP110 (HSP110wt) by IHC in MSI-H CRCs\textsuperscript{[96]} and found that reduced expression of HSP110wt was correlated with a large deletion in the *HSP110* T\textsuperscript{r} repeat and favorable prognosis in MSI-H CRCs, which is reasonable because the HSP110wt expression level is expected to be inversely correlated with the HSP110\textsuperscript{ΔE9} expression level. Mutation of *HSP110* and variation in HSP110 expression are representative of the molecular heterogeneity associated with the prognostic heterogeneity of MSI-H CRCs, and it is expected that these molecular alterations could be used as predictive markers and therapeutic targets in MSI-H CRCs\textsuperscript{[54,143]}.

**Beta2 microglobulin:** According to recent investigations, coding microsatellite mutations in the beta2 microglobulin (B2M) gene occur in approximately 30% of MSI-H CRCs and are significantly associated with a low risk of disease relapse and a low frequency of distant metastasis in MSI-H CRCs\textsuperscript{[54,143]}. Although the molecular mechanism underlying how B2M mutations affect prognosis in MSI-H CRCs is not fully understood, the biological functions of B2M may be associated with the metastatic potential of cancer cells, based on results demonstrating that B2M can induce epithelial-mesenchymal transition in cancer cells and may mediate bone metastasis of cancers\textsuperscript{[144]}. As a putative prognostic marker and potential therapeutic target, the functional and prognostic significance of B2M mutations in MSI-H CRCs should be further evaluated.

**Myosin 1a:** A recent study by Mazzolini et al\textsuperscript{[53]} reported that the brush border protein myosin 1a (MYO1A) could act as a tumor suppressor in the intestine, and frameshift mutations in the *MYO1A* gene were detected in 32% of MSI-H CRCs. Interestingly, according to this study, a low expression level of MYO1A was associated with worse survival in patients with MSI-H CRCs, and MYO1A expression was identified as an independent prognostic factor in MSI-H CRCs. However, there is a lack of data elucidating the biological and clinicopathological significance of reduced MYO1A expression in CRCs; additional experimental and clinical studies are therefore needed.

**CDX2:** CDX2 (caudal-type homeobox 2) and CK20 (cytokeratin 20) are proteins associated with intestinal differentiation and are also important markers of the normal intestinal epithelium and CRCs. Several previous studies identified that a loss of CDX2 and/or CK20 expression in CRCs was associated with MSI-H or CIMP-H status\textsuperscript{[24,145-148]}. In a recent investigation, we found that a loss of CDX2/CK20 expression was significantly associated with poor differentiation, CIMP-H status, and an unfavorable prognosis in MSI-H CRCs\textsuperscript{[149]}. According to our study, CRC patients with simultaneous loss of CDX2 and CK20 expression (positive CDX2/CK20) were associated with poor differentiation, CIMP-H status, and an unfavorable prognosis in MSI-H CRCs\textsuperscript{[149]}. According to this study, CRC patients with simultaneous loss of CDX2 and CK20 expression (positive CDX2/CK20) were associated with poor differentiation, CIMP-H status, and an unfavorable prognosis in MSI-H CRCs\textsuperscript{[149]}. According to this study, CRC patients with simultaneous loss of CDX2 and CK20 expression (positive CDX2/CK20) were associated with poor differentiation, CIMP-H status, and an unfavorable prognosis in MSI-H CRCs\textsuperscript{[149]}.

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**Table 3 Potential prognostic molecular factors for microsatellite instability-high colorectal cancer: A review of the literature**

| Molecular factors | Prognostic implication in MSI-H CRC (molecular alteration) | Ref. |
|-------------------|----------------------------------------------------------|-----|
| HSP110            | Favorable (high expression of mutant HSP110)             | Dorard et al\textsuperscript{[59]}, 2011 Kim et al\textsuperscript{[54]}, 2014 |
| Beta2 microglobulin| Favorable (mutation of beta2 microglobulin)              | Kloor et al\textsuperscript{[53]}, 2007 Titkicheva et al\textsuperscript{[59]}, 2012 Mazzolini et al\textsuperscript{[59]}, 2012 |
| Myosin 1a         | Unfavorable (low expression of myosin 1a)                | Kim et al\textsuperscript{[59]}, 2013 |
| CDX2/CK20         | Unfavorable (loss of CDX2/CK20 expression)               | Isaksson-Mettavainio et al\textsuperscript{[146]}, 2012 |
| SMAD4             | Favorable (high expression of SMAD4)                     | Bae et al\textsuperscript{[147]}, 2011 |
| CIMP              | Unfavorable (CIMP-H)                                     | Bae et al\textsuperscript{[147]}, 2011 |
| LINE-1            | Unfavorable (low LINE-1 methylation level)               | Rhee et al\textsuperscript{[148]}, 2012 |

CIMP: CpG island methylator phenotype; CIMP-H: CIMP-high; CRC: Colorectal cancer; LINE-1: Long interspersed nucleotide element-1; MSI-H: Microsatellite instability-high; HSP110: Heat shock protein 110 kDa; CDX2: Caudal-type homeobox 2; CK20: Cytokeratin 20; SMAD4: SMAD family member 4.
and CK20 expression in tumor tissue constituted a highly aggressive subgroup of MSI-H CRC patients, with early death or recurrence occurring in this subgroup. Although CDX2/CK20 loss is not a specific molecular alteration associated with MSI-H CRC, these molecular factors can likely be used as markers to classify patients with MSI-H CRCs into prognostic subgroups.

**SMAD4:** In a recent investigation by Isaksson-Mettävainio et al.\(^{[150]}\), high SMAD4 (SMAD family member 4) expression was significantly correlated with a favorable prognosis in MSI-H CRCs. Previous studies have also revealed that a loss of SMAD4 expression is associated with advanced stage, metastatic potential and an adverse prognosis in CRCs\(^{[151-154]}\). In fact, loss of SMAD4 has been shown to be associated with 18q LOH\(^{[154,155]}\) and may not be correlated with MSI status in CRCs because 18q LOH is a characteristic molecular alteration in the CIN pathway. Although it is thought that the prognostic implication of SMAD4 expression can be applied to all CRCs, a variation in SMAD4 expression and its significance for the prognosis of MSI-H CRCs remains an interesting field of study. Therefore, the underlying mechanism and prognostic value of variations in SMAD4 expression in MSI-H CRCs should be further explored.

**CIMP:** The prognostic value of CIMP status in CRCs remains unclear. A few previous studies reported that the CIMP-H subtype was associated with poor prognosis in patients with CRC; however, this adverse effect of CIMP-H on CRC prognosis was significant only in the MSS patient subgroup and not in the MSI-H patient subgroup\(^{[86,98]}\). However, Ogino et al.\(^{[4]}\) provided contrasting data showing that CIMP-H was associated with a low cancer-specific mortality in CRC patients, regardless of both MSI status and BR-4f mutations. In addition, although there is a lack of data elucidating the prognostic significance of CIMP-L in CRCs, a study by Dahlin et al.\(^{[86]}\) found that the CIMP-L subtype was associated with an unfavorable prognosis for CRCs, regardless of MSI status. The dependence of a chemotherapeutic response on CIMP status in CRCs is also controversial. Some investigators have suggested that CIMP-H is associated with a survival benefit in CRC patients receiving 5-FU-based chemotherapy\(^{[99,156]}\), whereas the opposite results have also been reported\(^{[157,158]}\). Focusing on the prognostic implication of CIMP for MSI-H CRCs, we previously reported that for MSI-H CRC patients, those with CIMP-H tumors had worse survival than those with CIMP-L/0 tumors\(^{[87]}\). In fact, it is suspected that the differences in survival according to CIMP status in patients with MSI-H CRC may reflect differences in age distribution; in particular, patients with sporadic MSI-H CRC, who are expected to have CIMP-H status, are older and may have various comorbidities. In contrast, those with Lynch syndrome CRC, who have a CIMP-L/0 status, are younger and may be relatively healthy. This age distribution may critically affect patient prognosis, and thus, the prognostic significance of CIMP status in MSI-H CRC may be partly influenced by this age effect. Currently, it is debated whether CIMP status can serve as a true prognostic factor for MSI-H CRCs; therefore, more attention must be paid to analyzing the prognostic and predictive effect of CIMP status in CRCs.

**LINE-1 methylation:** Several recent investigations have revealed that a low LINE-1 methylation level is independently associated with an adverse prognosis for CRCs\(^{[159-161]}\). According to one of our studies\(^{[162]}\), this prognostic significance of LINE-1 methylation was also
maintained in MSI-H CRCs. A low LINE-1 methylation level was an independent factor indicating poor prognosis in MSI-H CRC. These findings indicate that the LINE-1 methylation level can be a useful molecular factor for selecting the poor prognostic subgroup among patients with MSI-H CRC, which is known to be associated with a favorable prognosis, despite the level of LINE-1 hypomethylation being mild in MSI-H CRCs, as described above. 

**CONCLUSION**

MSI-H CRCs have been characterized as demonstrating a favorable prognosis and low benefit of adjuvant chemotherapy. However, MSI-H CRCs are molecularly heterogeneous tumors; thus, it is strongly suspected that prognostic and predictive molecular factors may be present. To date, several molecular factors, including HSP110, B2M, MYO1A, CDX2/CK20, SMAD4, CIMP, and LINE-1, have been explored as potential prognostic markers for MSI-H CRCs. However, additional investigations are necessary to identify the molecular determinants of patient prognosis and therapeutic response in MSI-H CRCs.

**REFERENCES**

1. Alitalo LA, Salovaara R, Kristo P, Canzian F, Hemminki A, Peltomäki P, Chadwick RB, Kääriäinen H, Eskelinen M, Järvinen 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 [PMID: 9595786 DOI: 10.1056/NEJM199805283382101]  
2. Salovaara R, Loukola A, Kristo P, Kääriäinen H, Ahtola H, Eskelinen M, Härkönen N, Julkunen R, Kangas E, Ojala S, Tulikoura J, Valkamo E, Järvinen H, Mecklin JP, Alitalo LA, de la Chapelle A. Population-based molecular detection of hereditary nonpolyposis colorectal cancer. **J Clin Oncol** 2000; 18: 2193-2200 [PMID: 10820038]  
3. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, Nakagawa H, Sotamai K, Prior TW, Westman J, Panescu J, Fix D, Lockman J, Conners J, de la Chapelle A. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). **N Engl J Med** 2005; 352: 1851-1860 [PMID: 15872200 DOI: 10.1056/NEJMoa043146]  
4. Ogino S, Nosho K, Kirkner GJ, Kawasaki T, Meyerhardt JA, Loda M, Giovannucci EL, Fuchs CS. CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. **Gut** 2009; 58: 90-96 [PMID: 18832519 DOI: 10.1136/gut.2008.155473]  
5. Jin HY, Liu X, Li VK, Ding Y, Yang B, Geng J, Lai R, Ding S, Ni M, Zhao R. Detection of mismatch repair gene germline mutation carrier among Chinese population with colorectal cancer. **BMJ Cancer** 2008; 8: 44 [PMID: 18257912 DOI: 10.1186/1471-2407-8-44]  
6. Oh JR, Kim DW, Lee HS, Lee HE, Lee SM, Jang JH, Kang SB, Ku JL, Jeong SY, Park JG. Microsatellite instability testing in Korean patients with colorectal cancer. **Fam Cancer** 2012; 11: 459-466 [PMID: 22669410 DOI: 10.1007/s10689-012-9536-4]  
7. Lynch HT, Lynch PM, Lanspa SJ, Snyder CL, Lynch JF, Boland CR. Review of the Lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. **Clin Genet** 2009; 76: 1-18 [PMID: 19659756 DOI: 10.1111/j.1399-0004.2009.01230.x]  
8. Pino MS, Chung DC. Microsatellite instability in the management of colorectal cancer. **Expert Rev Gastroenterol Hepatol** 2011; 5: 385-399 [PMID: 21651356 DOI: 10.1586/ehg.11.25]  
9. de la Chapelle A, Hampel H. Clinical relevance of microsatellite instability in colorectal cancer. **J Clin Oncol** 2010; 28: 3380-3387 [PMID: 20516444 DOI: 10.1200/JCO.2009.27.0652]  
10. 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: 5248-5257 [PMID: 982339]  
11. Jenkins MA, Hayashi S, O’Shea AM, Burgart LJ, Smyrk TC, Shimizu D, Waring PM, Ruszkiewicz AR, Pollet AF, Redston M, Barker MA, Baron JA, Casey GR, Dowty JG, Giles CG, Limburg P, Newcomb P, Young JP, Walsh MD, Thibodeau SN, Lindor NM, Lemarchand L, Gallinger S, Haile RW, Potter JD, Hopper JL, Jass JR. Pathology features in Bethesda guidelines predict colorectal cancer microsatellite instability: a population-based study. **Gastroenterology** 2007; 133: 48-56 [PMID: 17631310 DOI: 10.1053/j.gastro.2007.04.044]  
12. Gatalica Z, Torlakovice E. Pathology of the hereditary colorectal carcinoma. **Fam Cancer** 2008; 7: 15-26 [PMID: 17564815 DOI: 10.1007/s10689-007-9146-8]  
13. Weisenberger DJ, Siegmund KD, Campan M, Young J, Long TJ, Faasse MA, Kang GH, Widschwendter M, Weener D, Buchanan D, Koh H, Simms L, Barker M, Leggett B, Levine J, Kim M, French AJ, Thibodeau SN, Jass J, Haile R, Laird PW. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. **Nat Genet** 2006; 38: 787-793 [PMID: 16804544 DOI: 10.1038/ng1834]  
14. Deng G, Bell I, Cravely S, Gum J, Terdiman JP, Allen BA, Truta B, Sleisenger MH, Kim YS. BRAF mutation is frequently present in sporadic colorectal cancer with methylated hMLH1, but not in hereditary nonpolyposis colorectal cancer. **Clin Cancer Res** 2004; 10: 191-195 [PMID: 14734469]  
15. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. **J Clin Oncol** 2005; 23: 609-618 [PMID: 15695908 DOI: 10.1200/JCO.2005.01.086]  
16. Guastadisegni C, Colafranceschi M, Ottini L, Dogliotti E. Microsatellite instability as a marker of prognosis and response to therapy: a meta-analysis of colorectal cancer survival data. **Eur J Cancer** 2010; 46: 2788-2798 [PMID: 20627535 DOI: 10.1016/j.ejca.2010.05.009]  
17. Benatti P, Gafa R, Barana D, Marino M, Scarselli A, Pedroni M, Maestri I, Guerzoni L, Roncucci L, Menigatti M, Roncari B, Maffei S, Rossi G, Ponti G, Santini A, Losi L, Di Gregorio C, Oliani C, Ponz de Leon M, Lanza G. Microsatellite instability and familial colorectal cancer prognosis. **Clin Cancer Res** 2005; 11: 8332-8340 [PMID: 16322293 DOI: 10.1158/1078-0432.CCR-05-1030]  
18. Jover R, Zapater P, Castells A, Llor X, Andreu M, Cubiella J, Balaguer F, Sempere L, Nicola RM, Bujanda L, Reñé JM, Zaroca J, Morillas JD, Nicolás-Pérez D, Pons E, Payá A, Alenda C. The efficacy of adjuvant chemotherapy with 5-fluorouracil in colorectal cancer depends on the mismatch repair status. **Eur J Cancer** 2009; 45: 365-373 [PMID: 18722765 DOI: 10.1016/j.ejca.2008.07.016]  
19. Des Guetz G, Schischmanoff O, Nicolas P, Perret GY, Morere JF, Uzzan B. Does microsatellite instability predict the efficacy of adjuvant chemotherapy in colorectal cancer? A systematic review with meta-analysis. **Eur J Cancer** 2009; 45: 1890-1896 [PMID: 19427194 DOI: 10.1016/j.ejca.2009.04.018]  
20. Pritchard CC, Grady WM. colorectal cancer molecular biology moves into clinical practice. **Gut** 2011; 60: 116-129 [PMID: 20921207 DOI: 10.1136/gut.2009.206250]
Grady WM, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. Gastroenterology 2008; 135: 1079-1099 [PMID: 18773902 DOI: 10.1053/j.gastro.2008.07.066]

Samowitz WS, Albertson H, Herrick J, Levin TR, Sweeney C, Murtough MA, Wolf RK, Slattery ML. Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. Gastroenterology 2005; 129: 837-845 [PMID: 16143123 DOI: 10.1053/j.gastro.2005.06.020]

Ogino S, Cantor M, Kawasaki T, Brahmmandam M, Kirkner GJ, Weisenberger DJ, Campan M, Laird PW, Loda M, Fuchs CS. CpG island methylator phenotype (CIMP) of colorectal cancer is best characterised by quantitative DNA methylation analysis and prospective cohort studies. Gut 2006; 55: 1000-1006 [PMID: 16407376 DOI: 10.1136/gut.2005.082933]

Zlabec I, Bihl M, Foerster A, Rufle A, Lugli A. Comprehensive analysis of CpG island methylator phenotype (CIMP)-high, -low, and -negative colorectal cancers based on protein marker expression and molecular features. J Pathol 2011; 228: 336-343 [PMID: 21669972 DOI: 10.1002/path.2679]

Kim JH, Rhee YY, Bae JM, Kwon HJ, Cho NY, Kim MJ, Martin DI, Ward RL. Germline epimutation of MLH1 in individuals with an MLH1 epimutation in an ethnically diverse South African cohort. Clin Genet 2011; 80: 428-434 [PMID: 21375527 DOI: 10.1111/j.1399-0004.2011.01660.x]

Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology 2010; 138: 2073-2087.e3 [PMID: 20420947 DOI: 10.1053/j.gastro.2009.12.064]

Geiersbach KB, Samowitz WS. Microsatellite instability and colorectal cancer. Arch Pathol Lab Med 2011; 135: 1269-1277 [PMID: 21970492 DOI: 10.5858/arpa.2011-0035-RA]

Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012; 487: 330-337 [PMID: 22810696 DOI: 10.1038/nature11252]

Parsons R, Myeroff LL, Liu B, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B. Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colorectal cancer. Cancer Res 1995; 55: 5548-5550 [PMID: 7585632]

Rampino N, Yamamoto H, Ionov Y, Li Y, Sawai H, Reed JC, Peruchio M. Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. Science 1997; 275: 967-969 [PMID: 9200777]

Hempen PM, Zhang L, Bansal RK, Iacobuzio-Donahue CA, Murphy KM, Maitra A, Vogelstein B, Whitehead RH, Markowitz SD, Willson JK, Yeo CJ, Hruban RH, Kern SE. Evidence of selection for clones having genetic inactivation of the activin A type II receptor (ACVR2) gene in gastrointestinal cancers. Cancer Res 2003; 63: 994-999 [PMID: 12615714]

Calin GA, Gafa R, Biffi Ottone MG, Herlea V, Becheanu G, Cavazzini L, Barbanti-Brodano G, Nenci I, Negri M, Lanza G. Genetic progression in microsatellite instability in high (MSI-H) colon cancers correlates with clinico-pathological parameters: A study of the TGRbetaRII, BAX, hMSH3, hMSH6, IGFII and BLM genes. Int J Cancer 2000; 89: 230-235 [PMID: 10861498]

Ikeda M, Orimo H, Moriyama H, Nakajima E, Matsubara N, Mizu R, Tanaka N, Shimada T, Kimura A, Shimizu K. Close correlation between mutations of EP24 and hMSH3 genes in colorectal cancers with microsatellite instability. Cancer Res 1998; 58: 594-598 [PMID: 9485005]

Shin KH, Park YJ, Park JC. PTEN gene mutations in colorectal cancers displaying microsatellite instability. Cancer Lett 2001; 174: 189-194 [PMID: 11698295]

Woerner SM, Kloor M, Schwitalla Y, Youmans H, Doebertiz MV, Gebert J, Dilmann S. The putative tumor suppressor AIM2 is frequently affected by different genetic alterations in microsatellite unstable colon cancers. Genes Chromosomes Cancer 2007; 46: 1080-1089 [PMID: 17726700 DOI: 10.1002/gcc.20493]

Trojan J, Brieger A, Raedle J, Weber N, Kriener S, Kronenberg B, Caspary WF, Zeuzem S. BAX and caspase-5 frameshift mutations and spontaneous apoptosis in colorectal cancer with microsatellite instability. Int J Colorectal Dis 2004; 19: 538-544 [PMID: 15088110 DOI: 10.1007/s00384-004-0597-y]

Bader S, Walker M, Hendrich B, Bird A, Bird C, Hoofer M, Wylie A. Somatic frameshift mutations in the MBDA4 gene of sporadic colon cancers with mismatch repair deficiency. Oncogene 1999; 18: 8044-8047 [PMID: 10657515 DOI: 10.1038/sj.onc.1203299]

Duval A, Gayet J, Zhou XP, Iacopetta B, Thomas G, Hameelin R. Frequent frameshift mutations of the TCF-4 gene in colorectal cancers with microsatellite instability. Cancer Res 1999; 59: 4213-4215 [PMID: 10485457]

Nakagawa H, Koyama K, Nakamori S, Kameyama M, Imakura S, Monden M, Nakamura Y. Frameshift mutation of the STK11 gene in a sporadic gastrointestinal cancer with micro-
satellite instability. *Jpn J Cancer Res* 1999; 90: 633-637 [PMID: 10429655]

49 Kim NG, Choi YR, Baek MJ, Kim YH, Kang H, Kim NK, Min JS, Kim H. Frmsihift mutation at codon mononucleotide repeats of the hRad50 gene in gastrointestinal carcinomas with microsatellite instability. *Cancer Res* 2001; 61: 36-36 [PMID: 11196187]

50 Kim CJ, Lee JH, Song JW, Cho YG, Kim SY, Nam SW, Yoo NJ, Park WS, Lee JY. Chk1 frmsihift mutation in sporadic and hereditary non-polyposis colorectal cancers with microsatellite instability. *Eur J Surg Oncol* 2007; 33: 580-585 [DOI: 10.1016/j.ejso.2007.02.007]

51 Thorstensen L, Lind GE, Leving T, Diep CB, Melling GI, Rognum TO, Lothe RA. Genetic and epigenetic changes of components affecting the WNT pathway in colorectal carcinomas stratified by microsatellite instability. *Neoplasia* 2005; 7: 99-108 [PMID: 15802015 DOI: 10.1593/neo.044448]

52 Wicking C, Simms LA, Evans T, Walsh M, Chawengsakphak K, Beck F, Chennevix-Trench G, Young J, Jass J, Leggett B, Wainwright B. CDX2, a human homologue of Drosophila caudal, is mutated in both alleles in a replication error positive colorectal cancer. *Oncogene* 1998; 17: 657-659 [PMID: 9704932 DOI: 10.1038/sj.onc.1201971]

53 Mazzolini R, Dopeso F, Mateo-Lozano S, Sweeney C, Herrick J, Albertsen H, Levin Y, Liao X, Meyerhardt JA, Fuchs CS, Ogino S. TGFBR2 and BAX in neoplastic progression and patient outcome in sporadic colorectal tumors. *Neoplasia* 2007; 9: 1085-1094 [PMID: 17938166 DOI: 10.1593/ijn.06-1044]

54 Leochhead P, Kuchiba A, Imamura Y, Liu X, Yamashi T, Nishihara R, Qian ZR, Morikawa T, Sheng J, Meyerhardt JA, Fuchs CS, Ogino S. Microsatellite instability and BRAF mutation testing in colorectal cancer progression. *J Natl Cancer Inst* 2013; 105: 1151-1156 [PMID: 23878352 DOI: 10.1093/jnci/djt173]

55 De Roock W, Claes B, Bernasconi D, De Schutter J, Biesman B, Fouentzas G, Kalogeris KT, Kotoulà V, Papamichael D, Laurent-Puig P, Penna L, Roupie P, Vincenzi B, Santini D, Tonini G, Cappuzzo F, Frattini M, Molinari F, Saletti P, Do Dosso S, Martini M, Bardelli A, Siena S, Sartore-Bianchi A, Tabernero J, Macarulla T, Di Fiore F, Gangloff AO, Ciardelli F, Pfeiffer P, Qvortrup C, Hansen TP, Van Cutsem E, Piessevaux H, Lambert D, Delorenzi M, Teijpar S. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 2010; 11: 753-762 [PMID: 20619739 DOI: 10.1016/S1470-2045(10)70130-3]

56 Samowitz WS, Sweeney C, Herrick J, Albertsen H, Levin TR, Murtaugh MA, Wolff RK, Slattery ML. Poor survival associated with the BRAF V600E mutation in microsatellite stable colon cancers. *Cancer Res* 2005; 65: 6063-6069 [PMID: 16024606 DOI: 10.1158/0008-5472.CAN-05-0404]

57 Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kimosowid M, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chennevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cosco A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuan ST, Weber BL, Seigler HF, Qvortrup C, Hansen TP, Van Cutsem E, Piessevaux H, Lambert D, Delorenzi M, Teijpar S. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on survival, stage and histology in sporadic microsatellite unstable colon cancers. *Int J Cancer* 2006; 118: 2509-2513 [PMID: 16380996 DOI: 10.1002/ijc.21710]

58 Fernández-Peralta AM, Nejda N, Oliart S, Medina V, Azcoitia MM, González-Aguillera J. Significance of mutations in TGFBR2 and BAX in neoplastic progression and patient outcome in sporadic colorectal tumors with high-frequency microsatellite instability. *Cancer Genet Cytof genet* 2005; 157: 18-24 [PMID: 15676142 DOI: 10.1016/j.jcancergenct.2004.05.008]

59 Shina K, Morikawa T, Yamauchi M, Kuchiba A, Imamura Y, Liao X, Meyerhardt JA, Fuchs CS, Ogino S. TGFBR2 and BAX mononucleotide tract mutations, microsatellite instability, and prognosis in 1072 colorectal cancers. *PLoS One* 2011; 6; e25062 [PMID: 21949851 DOI: 10.1371/journal.pone.0025062]

60 Samowitz WS, Curtin K, Neuhausen S, Schaffer D, Slattery ML. Prognostic implications of BAX and TGFBR2I mutations in colon cancers with microsatellite instability. *Genes Chromosomes Cancer* 2002; 35: 368-371 [PMID: 12378532 DOI: 10.1002/gcc.10125]

61 Dorador C, de Thonel A, Collura A, Marisa L, Srveck M, Lagrange A, Jego G, Wanherdrick K, Joly AL, Buhard O, Gobbo J, Penard-Lacroix V, Zoauli H, Tubacher E, Kirzin S, Selves J, Milano G, Etienne-Grimaldi MC, Bengrine-Levère L, Louvet C, Tourmand C, Levère JH, Parc Y, Tiet E, Fléjou JF, Gaub MP, Garrido C, Duval A. Expression of a mutant HSP110 sensitizes colorectal cancer cells to chemotherapy and improves disease prognosis. *Nat Med* 2011; 17: 1283-1289 [PMID: 21946539 DOI: 10.1038/nm.2457]

62 Giannini G, Rinaldi C, Ristori E, Ambrosini ML, Cerignoli F, Viel A, Bidoli E, Berni S, D’Amati G, Scambia G, Frati L, Screpanti I, Gulino A. Mutations of an intron repeat induce impaired MRE11 expression in primary human cancer with microsatellite instability. *Oncogene* 2004; 23: 2640-2647 [PMID: 15048091 DOI: 10.1038/sj.onc.1207409]

63 Rajagopal H, Azzopardi L, Loewith R, Cizykova B, Kizler KW, Vogelstein B, Velculescu VE. Tumourigenesis: RAF/RAS oncogenes and mismatch-repair status. *Nature* 2002; 418: 934 [PMID: 12198537 DOI: 10.1038/418934a]

64 Siena S, Santoro-Bianchi A, Di Nicolantonio F, Balfour J, Bardelli A. Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer. *J Natl Cancer Inst* 2009; 101: 1308-1324 [PMID: 19738166 DOI: 10.1093/jnci/djp289]

65 Vaught CP, Zobell SD, Furtado LV, Baker CL, Samowitz WS. Frequency of KRAS, BRAF, and NRAS mutations in recurrent KRAS codon 146 mutations in human colorectal cancer. *Cancer Biol Ther* 2006; 5: 926-932 [PMID: 16969076]
Microsatellite-unstable CRC
cancer: possible associations with male sex and female age.
In Colorectal Dis 2011; 13: 554-560 [PMID: 21915755 DOI: 10.1111/j.1365-2362.2011.02267.x]

82 Hawkins N, Norrie M, Cheung L, Mokany E, Ku SL, Meagher A, Levine T, Ward R. CpG island methylation in sporadic colorectal cancers and its relationship to microsatellite instability. Gastroenterology 2002; 122: 1376-1387 [PMID: 11984524]

79 Samowitz WS, Albertsen H, Sweeney C, Herrick J, Caan BJ, Anderson KE, Wolf RK, Slattery ML. Association of smoking, CpG island methylator phenotype, and V600E BRAF mutations in colon cancer. J Natl Cancer Inst 2006; 98: 1751-1758 [PMID: 17148775 DOI: 10.1093/jnci/dj648]

78 Barault L, Charon-Barra C, Jooste V, de la Vega MF, Martin L, Roignon P, Rat P, Bouvier AM, Laurent-Puig P, Fairev J, Chapusot C, Piard F. Hypermethylator phenotype in sporadic colon cancer: study on a population-based series of 582 cases. Cancer Res 2008; 68: 8541-8546 [PMID: 18922929 DOI: 10.1158/0008-5472.CAN-08-1171]

77 Dahlin AM, Palmqvist R, Henriksson ML, Jacobsson M, Estécio MR, Doshi K, Kondo Y, Tajara EH, Issa JP. CpG island methylator phenotype in colorectal cancer. Gastroenterology 2006; 130: 1792-1803 [PMID: 16582178 DOI: 10.1053/j.gastro.2006.01.068]

76 Grossmann AH, Samowitz WS. Epidermal growth factor receptor pathway mutations and colorectal cancer therapy. Arch Pathol Lab Med 2011; 135: 1278-1282 [PMID: 21970483 DOI: 10.5888/aplum.2011-0047-RA]

75 Loughrey MB, Waring PM, Tan A, Zeuzem S, Raelder J. BRAF mutations in colorectal carcinoma suggest two entities of microsatellite-unstable tumors. Cancer 2003; 104: 952-961 [PMID: 16015629 DOI: 10.1002/cncr.11266]

74 Domingo E, Lahno P, Ollikainen M, Pinto M, Wang L, French AJ, Westra J, Frebourg T, Espin E, Armengol M, Hamelin R, Yamamoto H, Hoßftra RM, Seruca R, Lindblom A, Pettermäki P, Thibodeau SN, Aaltonen LA, Schwartz S. BRAF screening as a low-cost effective strategy for simplifying HNPPC genetic testing. J Med Genet 2004; 41: 664-668 [PMID: 15342696 DOI: 10.1136/jmg.2004.020651]

73 Lumbroiski N, Plotz G, Wormek M, Engels K, Kriener S, Trojan J, Jungling B, Zeuzem S, Raelder J. BRAF mutations in colorectal carcinoma suggest two entities of microsatellite-unstable tumors. Cancer 2003; 104: 952-961 [PMID: 16015629 DOI: 10.1002/cncr.11266]

72 Grossmann AH, Samowitz WS. Epidermal growth factor receptor pathway mutations and colorectal cancer therapy. Arch Pathol Lab Med 2011; 135: 1278-1282 [PMID: 21970483 DOI: 10.5888/aplum.2011-0047-RA]

71 Janakiram M, Vakiani E, Zeng Z, Pratillas CA, Taylor BS, Chitale D, Hallock E, Wilson M, Huberkan B, Ricarte Filho JC, Persaud Y, Levine DA, Faig J, Jhamwar S, Mariadason JM, Lash A, Ladanyi M, Salz LB, Heguy A, Paty PB, Solit DB. Genomic and biological characterization of exon 4 KRAS mutations in human cancer. Cancer Res 2010; 70: 5901-5911 [PMID: 20570890 DOI: 10.1158/0008-5472.CAN-10-1092]

70 Suter CM, Martin DI, Ward RL. Hypomethylation of L1 in colorectal adenomas and cancers of the colorectum. J Med Genet 2004; 41: 664-668 [PMID: 15342696 DOI: 10.1136/jmg.2004.020651]

69 Tadesse Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel OD, Van de Velde CJ, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel OD. Differential expression of CXCR4 and its chemokine ligands in colorectal cancer: possible associations with male sex and female age. J Mol Diagn 2006; 8: 582-588 [PMID: 17065427 DOI: 10.2353/jmoldx.2006.060802]

68 Kim JH, Walsh MD, Barker MA, Arnold S, Simms LA, Leggett BA, Fayers P, Persaud Y, Levine T, Ward R. CpG island methylation in sporadic colorectal cancers and its relationship to microsatellite instability. Gastroenterology 2002; 122: 1376-1387 [PMID: 11984524]

67 Samowitz WS, Albertsen H, Sweeney C, Herrick J, Caan BJ, Anderson KE, Wolf RK, Slattery ML. Association of smoking, CpG island methylator phenotype, and V600E BRAF mutations in colon cancer. J Natl Cancer Inst 2006; 98: 1751-1758 [PMID: 17148775 DOI: 10.1093/jnci/dj648]
Microsatellite-unstable CRC

Kim JH et al. 2014;11:856-862 | DOI: 10.1158/1078-0432.CCR-13-0065

Kumar K, Brim H, Giardiello F, Smoot DT, Nouraie M, Lee AH, As borough K, Distinct BRAF (V600E) and KRAS mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial. J Clin Oncol 2009; 27: 5031-5037 | DOI: 10.1200/JCO.2009.22.2425

Kumar K, Brim H, Giardiello F, Smoot DT, Nouraie M, Lee AH, Ashbrook K. Distinct BRAF (V600E) and KRAS mutations in high microsatellite instability sporadic colorectal cancer in African Americans. Clin Cancer Res 2009; 15: 1155-1161 | PMID: 19910929 DOI: 10.1158/1078-0432.CCR-08-1029

Vilkin A, Niv Y, Nagasaka T, Morgenstern S, Levi Z, Fireman Z, Fuerst F, Goel A, Boland CR. Microsatellite instability, MLH1 promoter methylation, and BRAF mutation analysis in sporadic colorectal cancers of different ethnic groups in Israel. Cancer 2009; 115: 760-769 | PMID: 19127559 DOI: 10.1002/cncr.24019

Tie J, Gibbs P, Lipton L, Christie M, Jorissen RN, Burgess AW, Croxford M, Jones I, Langland R, Kosmider S, McKay D, Bollag G, Nolop K, Sieber OM, Desai J. Optimizing targeted therapeutic development: analysis of a colorectal cancer patient population with the BRAF(V600E) mutation. Int J Cancer 2011; 128: 2075-2084 | PMID: 20635392 DOI: 10.1002/ijc.25555

Yamachi M, Morikawa T, Kuchiba A, Imamura Y, Qian ZR, Nishihara R, Liao X, Waldron L, Hoshida Y, Huttonhower C, Chan AT, Giovannucci E, Fuchs C, Ogin S. Assessment of colorectal cancer molecular features along bowel subites challenges the conception of distinct dichotomy of proximal versus distal colorectum. Gut 2012; 61: 847-854 | PMID: 22427238 DOI: 10.1136/gutjnl-2011-300865

Kalady MF, Dejulius KL, Sanchez JA, Jarrar A, Liu X, Millich E, Skakel M, Church JM. BRAF mutations in colorectal cancer are associated with distinct clinical characteristics and worse prognosis. Dis Colon Rectum 2012; 55: 128-133 | PMID: 22292154 DOI: 10.1097/DCR.0b013e318256f881

Tian S, Simon I, Moreno V, Roepman P, Tabernero J, Snel M, van’t Veer L, Salazar R, Bernards R, Capella G. A combined oncogenic pathway signature of BRAF, KRAS and PIK3CA mutation improves colorectal cancer classification and cetuximab treatment prediction. Gut 2013; 62: 540-549 | PMID: 22798500 DOI: 10.1136/gutjnl-2012-302423

Nagasaka T, Satsomo H, Notohara K, Cullings HM, Takeda M, Kimura K, Kambara T, MacPhee DG, Young J, Leggett BA, Jass JR, Tanaka N, Matsushita N. Colorectal cancer with mutation in BRAF, KRAS, and wild-type with respect to both oncosgenes showing different patterns of DNA methylation. J Clin Oncol 2004; 22: 4584-4594 | PMID: 15542810 DOI: 10.1200/JCO.2004.02.154

Chang SC, Lin JK, Yang SH, Wang HS, Li AF, Chi CW. Relationship between genetic alterations and prognosis in sporadic colorectal cancer. Int J Cancer 2006; 118: 1721-1727 | PMID: 16233136 DOI: 10.1002/jic.21563

Yagi K, Akagi K, Hayashi H, Nagae G, Tsuji S, Isagawa T, Midorikawa Y, Nishimura Y, Sakamoto H, Seto Y, Aburatani H, Kameda A. Three DNA methylation epigenotypes in human colorectal cancer. Clin Cancer Res 2010; 16: 21-33 | PMID: 20028768 DOI: 10.1158/1078-0432.CCR-09-2006

Shen H, Yuan Y, Hu HG, Zhong X, Ye XX, Li MD, Fang WJ, Zheng S. Clinical significance of K-ras and BRAF mutations in Chinese colorectal cancer patients. World J Gastroenterol 2011; 17: 809-816 | PMID: 21390154 DOI: 10.3748/wjg.v17.i6.809

Liou JM, Wu MS, Shun CT, Chiu HM, Chen MJ, Chen CC, Wang HP, Lin JT, Liang JT. Mutations in BRAF correlate with poor survival of colorectal cancers in Chinese population. Int J Colorectal Dis 2011; 26: 1387-1395 | PMID: 21553007 DOI: 10.1007/s00384-011-1229-1

Yokota T, Ura T, Shibata N, Takahari D, Shibata K, Nomura M, Kondo C, Mizota A, Utsunomiya S, Muro K, Yatabe Y. BRAF as a potential prognostic factor in advanced and recurrent colorectal cancer. Br J Cancer 2011; 104: 856-862 | PMID: 21285991 DOI: 10.1038/bjc.2011.19

Aoyagi H, Iida S, Uetake H, Ishikawa T, Takagi Y, Kobayashi H, Higuchi T, Yasumo M, Enomoto M, Sugihara H. Effect of classification based on combination of mutation and methylation in colorectal cancer prognosis. Oncol Rep 2011; 25: 789-794 | PMID: 21174064 DOI: 10.3892/or.2010.1118

Hsieh LL, Er TK, Chen CC, Hsieh JS, Chang JG, Liu TC. Characteristics and prevalence of KRAS, BRAF, and PIK3CA mutations in colorectal cancer by high-resolution melting analysis in Taiwanese population. Clin Chim Acta 2012; 413: 1605-1611 | PMID: 22579930 DOI: 10.1016/j.cca.2012.04.029

www.wjgnet.com
Nakanishi R, Harada J, Tuul M, Zhao Y, Ando K, Saeki H, Oki E, Ohga T, Kito A, Kakeji Y, Maehara Y. Prognostic relevance of KRAS and BRAF mutations in Japanese patients with colorectal cancer. *Int J Clin Oncol* 2013; 18: 1042-1048 [PMID: 23586744 DOI: 10.1007/s10147-013-0992-1]

Kim GP, Colangelo LH, Wierand HS, Paik S, Kirsch IR, Wolmark N, Allegra CJ. 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: 767-772 [PMID: 17228025 DOI: 10.1200/JCO.2006.05.8172]

Kim GP, Colangelo LH, Paik S, O’Connell MJ, Kirsch IR, Allegra C, Wolmark N. Predictive value of microsatellite instability-high remains controversial. *J Clin Oncol* 2007; 25: 4857-4858 [PMID: 17947740 DOI: 10.1200/JCO.2007.13.2019]

Des Guet G, Uzzan B, Nicolas P, Schischmannoff O, Perret GY, Morere JF. Microsatellite instability does not predict the efficacy of chemotherapy in metastatic colorectal cancer. A systematic review and meta-analysis. *Anticancer Res* 2009; 29: 1615-1620 [PMID: 19443375]

Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, French AJ, Kabat B, Foster NR, Torri V, Ribic C, Grothey A, Moore M, Zaninoni A, Seitz JF, Sinicrope F, Gallinger S. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J Clin Oncol* 2010; 28: 3219-3226 [PMID: 20498939 DOI: 10.1200/JCO.2009.27.1825]

Carethers JM, Smith EJ, Behling CA, Nguyen L, Tajima A, Doctolero RT, Cabrera BL, Goel A, Arnold CA, Miyai K, Boldt CR. Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer. *Gastroenterology* 2004; 126: 394-401 [PMID: 14762775]

Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, Hamilton SR, Laurent-Puig P, Gryfe HC, Yun SH, Lee WY, Chun HK, Park YS. Clinical impact of microsatellite instability in colon cancer following adjuvant FOLFOX chemotherapy. *Clin Cancer Res* 2011; 17: 7470-7478 [PMID: 21998335 DOI: 10.1158/1078-0432.CCR-11-1048]

Han SW, Lee HJ, Bae JM, Cho NY, Lee KH, Kim TY, Oh DY, Im SA, Bang YJ, Jeong SY, Park KJ, Park JG, Kang GH, Kim TY. Methylation and microsatellite status and recurrence following adjuvant FOLFOX in colorectal cancer. *Int J Cancer* 2013; 132: 2209-2216 [PMID: 23034738 DOI: 10.1002/ijc.28788]

Kim ST, Lee J, Park SH, Park JO, Lim HY, Kang WK, Kim JY, Kim YH, Chang DK, Rheo PL, Kim DS, Yun H, Cho YB, Kim HC, Yun SH, Lee WY, Chun HK, Park YS. Identification of microsatellite instability in colorectal cancer patients treated with adjuvant FOLFOX chemotherapy. *Clin Cancer Res* 2010; 16: 659-667 [PMID: 20338121 DOI: 10.1158/1078-0432.2010-2064]

Li P, Fang YJ, Li F, Ou QJ, Chen G, Ma G. ERCC1, defective mismatch repair status as a prognostic biomarker of survival for stage III colorectal cancer. *Am J Surg* 2011; 202: 586-592 [PMID: 21036755]

Chan AT. Turning up the heat on colorectal cancer. *Nat Med* 2011; 17: 1186-1188 [PMID: 21988992 DOI: 10.1038/nm.2500]

Tikidzhieva A, Benner A, Michel S, Formentini A, Link KH, Dippold W, von Knebel Doeberitz M, Kloor M. Prognostic impact of microsatellite instability in colorectal cancer patients treated with adjuvant FOLFOX. *Anticancer Res* 2010; 30: 4297-4301 [PMID: 21036755]

Jossion S, Nomura T, Lin JT, Huang WC, Wu D, Zhou HA, Zayazafoon M, Weizmann MN, Gururajan M, Chung LW. p2 – microglobulin induces epithelial to mesenchymal transition and confers cancer lethality and bone metastasis in human cancer cells. *Cancer Res* 2011; 71: 2600-2610 [PMID: 21427356 DOI: 10.1158/0008-5472.CAN-10-3382]

Hinou T, Tani M, Lucas FC, Caca K, Dunn RL, Macri E, Loda M, Appelman HD, Cho KR, Fearon ER. Loss of CDX2 expression and microsatellite instability are prominent features of large cell minimally differentiated carcinomas of the colon. *Am J Pathol* 2001; 159: 2239-2248 [PMID: 11733373 DOI: 10.1016/S0002-9440(10)63074-X]

McGregor DK, Wu TT, Rashid A, Luthra R, Hamilton SR. Reduced expression of cytokeratin 20 in colorectal carcinomas with high levels of microsatellite instability. *Am J Surg Pathol* 2004; 28: 712-718 [PMID: 15166663]

Fellak D, Borrini F, Boive V, Viguier J, Jacob S, Miquel C, Sabourin JC, Ducrues 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: 7557-7566 [PMID: 12982994]

Vilar E, Scalltriti M, Balmaña J, Saura C, Guzman M, Arribas J, Baselga J, Tabernero J. Microsatellite instability due to hMLH1 deficiency is associated with increased cytotoxicity to irinotecan in human colorectal cancer cell lines. *Br J Cancer* 2008; 99: 1607-1612 [PMID: 18941461 DOI: 10.1038/sj.bjc.6604691]

Rodriguez R, Hansen LT, Phear G, Scroah J, Spang-Thomsen M, Cox A, Helladelphia T, Muthum M. Thymidine selectively enhances growth suppressive effects of camptothecin/irinotecan in MSI+ cells and tumors containing a mutation of MRE11. *Clin Cancer Res* 2008; 14: 5476-5483 [PMID: 18765539 DOI: 10.1158/1078-0432.CCR-08-0274]
Lugli A, Tzankov A, Zlobec I, Terracciano LM. Differential diagnostic and functional role of the multi-marker phenotype CDX2/CK20/CK7 in colorectal cancer stratified by mismatch repair status. Mod Pathol 2008; 21: 1403-1412 [PMID: 18587233 DOI: 10.1038/modpathol.2008.117]

Baba Y, Nosho K, Shima K, Freed E, Irahara N, Philips J, Meyerhardt JA, Hornick JL, Shivdasani RA, Fuchs CS, Ogino S. Relationship of CDX2 loss with molecular features and prognosis in colorectal cancer. Clin Cancer Res 2009; 15: 4665-4673 [PMID: 19584150 DOI: 10.1186/1078-0432.CCR-09-0401]

Kim JH, Rhee YY, Bae JM, Cho NY, Kang GH. Loss of CDX2/CK20 expression is associated with poorly differentiated carcinoma, the CpG island methylator phenotype, and adverse prognosis in microsatellite-unstable colorectal cancer. Am J Surg Pathol 2013; 37: 1532-1541 [PMID: 24025523 DOI: 10.1097/PAS.0b013e31829ab1c1]

Isaksson-Mettävainio M, Palmqvist R, Dahlin AM, Van Guelpen B, Rutegård J, Oberg A, Henriksson ML. High SMAD4 levels appear in microsatellite instability and hypermethylated colon cancers, and indicate a better prognosis. Int J Cancer 2012; 131: 779-788 [PMID: 21964812 DOI: 10.1002/ijc.26473]

Alazzouzi H, Alhopuro P, Salovaara R, Salmikorpi H, Järvinen H, Mecklin JP, Hemminki A, Schwartz S, Aaltonen LA, Arango D. SMAD4 as a prognostic marker in colorectal cancer. Clin Cancer Res 2005; 11: 2606-2611 [PMID: 15814640 DOI: 10.1158/1078-0432.CCR-04-1458]

Tanaka T, Watanabe T, Kazama Y, Tanaka J, Kanazawa T, Kazama S, Nagawa H. Loss of Smad4 protein expression and 18qLOH as molecular markers indicating lymph node metastasis in colorectal cancer—A study matched for tumor depth and pathology. J Surg Oncol 2008; 97: 69-73 [PMID: 17786972 DOI: 10.1002/jso.20896]

Tanaka T, Watanabe T, Kazama Y, Tanaka J, Kanazawa T, Kazama S, Nagawa H. Chromosome 18q deletion and Smad4 protein inactivation correlate with liver metastasis; A study matched for T- and N-classification. Br J Cancer 2006; 95: 1562-1567 [PMID: 17088901 DOI: 10.1038/sj.bjc.6603460]

Miyaki M, Iijima T, Konishi M, Sakai K, Ishii A, Yasuno M, Hishima T, Koike M, Shiita N, Iwama T, Utsunomiya J, Kuroki T, Mori T. Higher frequency of Smad4 gene mutation in human colorectal cancer with distant metastasis. Oncogene 1999; 18: 3098-3103 [PMID: 10340381 DOI: 10.1088/sj.onc.1202642]

Thiagalingam S, Lengauer C, Leach FS, Schutte M, Hahn SA, Overhauser J, Willson JK, Markowitz S, Hamilton SR, Kern SE, Kinzler KW, Vogelstein B. Evaluation of candidate tumour suppressor genes on chromosome 18 in colorectal cancers. Nat Genet 1996; 13: 343-346 [PMID: 8673134 DOI: 10.1038/ng0796-343]

Van Rijnsoever M, Elseah H, Joseph D, McCaul K, Iacopetta B. CpG island methylator phenotype is an independent predictor of survival benefit from 5-fluorouracil in stage III colorectal cancer. Clin Cancer Res 2003; 9: 2098-2103 [PMID: 1291294]

Jover R, Nguyen TF, Pérez-Carbonell L, Zapater P, Payá A, Alenda C, Rojas E, Cubiella J, Balaguer F, Morillas JD, Clofent J, Bujanda L, Rehé M, Bessa X, Nicola RM, Nicolás-Pérez D, Castells A, Andreu M, Llor X, Boland CR, Goel A. 5-Fluorouracil adjuvant chemotherapy does not increase survival in patients with CpG island methylator phenotype colorectal cancer. Gastroenterology 2011; 140: 1174-1181 [PMID: 2185836 DOI: 10.1053/j.gastro.2010.12.035]

Shen L, Catalano P, Benson AB, O’Dwyer P, Hamilton SR, Issa JP. Association between DNA methylation and shortened survival in patients with advanced colorectal cancer treated with 5-fluorouracil based chemotherapy. Clin Cancer Res 2007; 13: 6093-6098 [PMID: 17947473 DOI: 10.1158/1078-0432.CCR-07-1011]

Ahn JB, Chung WB, Maeda O, Shin SJ, Kim HS, Chung HC, Kim NK, Issa JP. DNA methylation predicts recurrence from resected stage III proximal colon cancer. Cancer 2011; 117: 1847-1854 [PMID: 21509761 DOI: 10.1002/cncr.227637]

Baba Y, Huttenhower C, Nosho K, Tanaka N, Shima K, Hazra A, Schernhammer ES, Hunter DJ, Giovannucci EL, Fuchs CS, Ogino S. Epigenomic diversity of colorectal cancer indicated by LINE-1 methylation in a database of 869 tumors. Mol Cancer 2010; 9: 125 [PMID: 20507599 DOI: 10.1186/1476-4598-9-125]

Ogino S, Nosho K, Kirkner GJ, Kawasaki T, Chan AT, Schernhammer ES, Giovannucci EL, Fuchs CS. A cohort study of tumoral LINE-1 hypomethylation and prognosis in colon cancer. J Natl Cancer Inst 2008; 100: 1734-1738 [PMID: 19033568]

Rhee YY, Kim MJ, Bae JM, Koh JM, Cho NY, Juhun YS, Kim D, Kang GH. Clinical outcomes of patients with microsatellite-unstable colorectal carcinomas depend on L1 methylation level. Ann Surg Oncol 2012; 19: 3441-3448 [PMID: 22618722 DOI: 10.1245/s10434-012-2410-7]

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