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TOPIC HIGHLIGHT

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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.

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[1-6]. 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 called hereditary nonpolyposis 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[7,8]. 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[9].

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[10]. 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[4,11-14]. 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[15,16], whereas the beneficial effect of 5-fluorouracil (5-FU)-based adjuvant chemotherapy in patients with MSI-H CRC has been controversial[15-19]. 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[20]. 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[21]. 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[4,22-24]. 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[25]. 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[26,27]. 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[28,29]. In addition, constitutional epimutation of the *MLH1* gene has also been found in a few patients with Lynch syndrome[30-34]. Although it has been reported that an association between *MLH1* epimutation and a family history of CRC is not evident[30], the clinical and pathological significance of epimutations in MMR genes in hereditary MSI-H CRCs

remains unclear. According to our data, *MLH1* methylation-positive/CIMP-negative tumors account for 7.3% of MSI-H CRCs[25], 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 MutSα and MutLα complexes. The MutSα complex comprises a MSH2-MSH6 heterodimer, whereas the dimer of MLH1 and PMS2 forms the MutLα 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 MMR 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*, *CASPASE5*, *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[55,56], 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[59,60]. In the recent study by Dorard *et al*[59], deletions of the T_{17} 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 *RAF* kinase 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

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*[22], 2005	United States	250/859 (29.1)	64/78 (82.1)
Weisenberger *et al*[13], 2006	United States	33/187 (17.6)	NA
Samowitz *et al*[84], 2006	United States	313/1271 (24.6)	105/170 (61.8)
Barault *et al*[85], 2008	France	95/578 (16.4)	58/80 (72.5)
Ogino *et al*[4], 2009	United States	123/631 (19.5)	86/118 (72.9)
Dahlin *et al*[86], 2010	Sweden	46/411 (11.2)	34/61 (55.7)
Zlobec *et al*[24], 2011	Switzerland	22/314 (7)	NA
Lochhead *et al*[63], 2013	United States	205/1173 (17.5)	140/184 (76.1)
Eastern countries			
Koinuma *et al*[101], 2004	Japan	NA	16[1]/28 (57.1)
Nagasaka *et al*[102], 2008	Japan and Germany	NA	15[2]/36 (41.7)
Kim *et al*[98], 2009	South Korea	29/271 (10.7)	8/33 (24.2)
Min *et al*[99], 2011	South Korea	34/245 (13.9)	NA
Bae *et al*[97], 2013	South Korea	47/734 (6.4)	18/65 (27.7)
Kim *et al*[25], 2013	South Korea	NA	64/220 (29.1)

[1]*MLH1*-methylated colorectal cancers (CRCs) instead of CpG island methylator phenotype-high (CIMP-H) CRCs; [2]Sporadic 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[61,62]. According to previous studies, *BRAF* mutations were found in 5%-15% of overall CRCs[61,63-66], whereas 32%-40% of CRCs have *KRAS* mutations[63,64,67-72]. 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[36]. 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[63,73-76]. 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[14,73,77]. 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[13,23]. Regarding the implications for prognosis, several previous studies have reported that *BRAF* mutations indicate poor survival in patients with CRC[4,78]. 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[65,79]. Therefore, the clinical and prognostic significance of *BRAF* mutations in MSI-H CRCs should be carefully explored in larger samples.

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[80]. The CIMP status of CRCs can be classified into three subtypes: CIMP-H, CIMP-low (CIMP-L), and CIMP-zero (CIMP-0)[24,81,82]. 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[4,13,83]. 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)[4,13,22,24,63,84-86]. 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[87]. The effect of CIMP status on MSI-H CRC prognosis will be 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[88,89]. 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[90]. Interestingly, several previous investigations have reported that LINE-1 hypomethylation is inversely correlated with MSI-H status in CRCs[91-93]. In a study by Ogino *et al*[93], 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[8,9,35]. However, according to previous studies reported by our

Table 2 Frequency of *BRAF* V600E mutations in colorectal cancers: A review of the literature

Ref.	Country	*BRAF*-mutant CRCs/tested CRCs *n* (%)	
		In all CRCs	In MSI-H CRCs
Western countries			
Samowitz *et al*[22], 2005	United States	86/859 (10)	43/78 (55.1)
Weisenberger *et al*[13], 2006	United States	26/187 (13.9)	NA
Samowitz *et al*[84], 2006	United States	123/1271 (9.7)	67/170 (39.4)
Goel *et al*[103], 2007	United States	26/126 (20.6)	17/24 (70.8)
Maestro *et al*[104], 2007	Spain	12/324 (3.7)	9/49 (18.4)
Barault *et al*[85], 2008	France	76/578 (13.1)	51/80 (63.8)
French *et al*[105], 2008	United States	77/490 (15.7)	35/58 (60.3)
Ogino *et al*[4], 2009	United States	105/631 (16.6)	53/118 (44.9)
Richman *et al*[106], 2009	United Kingdom	56/710 (7.9)	NA
Kumar *et al*[107], 2009	United States (African)	7/98 (7.1)	7/30 (23.3)
Vilkin *et al*[108], 2009	Israel	24/128 (18.8)	6/13 (46.2)
Roth *et al*[79], 2010	Europe	103/1307 (7.9)	45/188 (23.9)
Dahlin *et al*[86], 2010	Sweden	55/411 (13.4)	34/61 (55.7)
Zlobec *et al*[24], 2011	Switzerland	42/314 (13.4)	NA
Tie *et al*[109], 2011	Australia	52/525 (9.9)	24/75 (32)
Yamauchi *et al*[110], 2012	United States	183/1276 (14.3)	NA
Kalady *et al*[111], 2012	United States	56/475 (11.8)	29/76 (38.2)
Tian *et al*[112], 2013	The Netherland and Spain	42/381 (11)	NA
Lochhead *et al*[63], 2013	United States	182/1253 (14.5)	101/193 (52.3)
Eastern countries			
Koinuma *et al*[101], 2004	Japan	16/140 (11.4)	12/28 (42.9)
Nagasaka *et al*[113], 2004	Japan and Australia	21/234 (9)	16/35 (45.7)
Chang *et al*[114], 2006	Taiwan	9/213 (4.2)	7/19 (36.8)
Nagasaka *et al*[102], 2008	Japan and Germany	20/243 (8.2)	10/36 (27.8)
Kim *et al*[98], 2009	South Korea	13/271 (4.8)	3/33 (9.1)
Yagi *et al*[115], 2010	Japan	13/149 (8.7)	NA
Shen *et al*[116], 2011	China	2/118 (1.7)	NA
Liou *et al*[117], 2011	Taiwan	12/314 (3.8)	NA
Yokota *et al*[118], 2011	Japan	15/229 (6.6)	NA
Aoyagi *et al*[119], 2011	Japan	1/134 (0.7)	NA
Kwon *et al*[100], 2011	South Korea	4/92 (4.3)	NA
Min *et al*[99], 2011	South Korea	11/245 (4.5)	6/49 (12.2)
Hsieh *et al*[120], 2012	Taiwan	2/182 (1.1)	NA
Nakanishi *et al*[121], 2012	Japan	17/254 (6.7)	11/31 (35.5)
Bae *et al*[97], 2013	South Korea	39/728 (5.4)	4/65 (6.2)
Kim *et al*[25], 2013	South Korea	NA	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 *BRAF* 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 *BRAF* 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)[4,13,22,24,63,84-86], 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)[25,97-99,101,102]. In Western countries, the *BRAF* 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)[25,97-102,113-121]. These findings suggest that the Eastern Asian ethnicity is associated with a relatively low prevalence of CIMP-H and *BRAF* 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 *BRAF* 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[18,122], it has been consistently reported that patients with MSI-H CRC show a better survival than MSI-L/MSS CRC patients[15,16]. However, there has been controversy regarding the predictive value of MSI status for the response to adjuvant chemotherapy in patients with CRC[122-124]. 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[132], several studies supporting MSI-H as a predictive factor for improved response to irinotecan or irinotecan-based chemotherapy in CRC patients have been reported[133,134]. In previous experiments, it has also been suggested that mutations of *MRE11* and/or *RAD50* found in MSI-H CRC cells could account for increased sensitivity to irinotecan[135,136]. 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

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/low expression of wild-type HSP110)	Dorard *et al*[59], 2011 Kim *et al*[96], 2014
Beta2-microglobulin	Favorable (mutation of beta2-microglobulin)	Kloor *et al*[54], 2007 Tikidzhieva *et al*[143], 2012
Myosin 1a	Unfavorable (low expression of myosin 1a)	Mazzolini *et al*[53], 2012
CDX2/CK20	Unfavorable (loss of CDX2/CK20 expression)	Kim *et al*[149], 2013
SMAD4	Favorable (high expression of SMAD4)	Isaksson-Mettavainio *et al*[150], 2012
CIMP	Unfavorable (CIMP-H)	Bae *et al*[87], 2011
LINE-1	Unfavorable (low LINE-1 methylation level)	Rhee *et al*[162], 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.

survival in CRC patients who are treated with adjuvant FOLFOX[137], whereas other studies have reported that MSI status was not significantly associated with a survival benefit of CRC patients after adjuvant FOLFOX treatment[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*[59] recently reported that the expression level of mutant HSP110 (heat shock protein 110 kDa) is significantly associated with prognosis and chemotherapy response in MSI-H CRCs. According to this study, the T_{17} 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 (HSP110ΔE9). MSI-H CRC patients with a high mRNA expression level of HSP110ΔE9 survived longer, and this improved survival was maintained in both stage Ⅲ and adjuvant chemotherapy-treated subgroups[59]. In our recent study, we evaluated the expression status of wild-type HSP110 (HSP110wt) by IHC in MSI-H CRCs[96] and found that reduced expression of HSP110wt was correlated with a large deletion in the *HSP110* T_{17} repeat and favorable prognosis in MSI-H CRCs, which is reasonable because the HSP110wt expression level is expected to be inversely correlated with the HSP110ΔE9 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[142].

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[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[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*[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/CK20: 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[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[149]. According to our study, CRC patients with simultaneous loss of CDX2

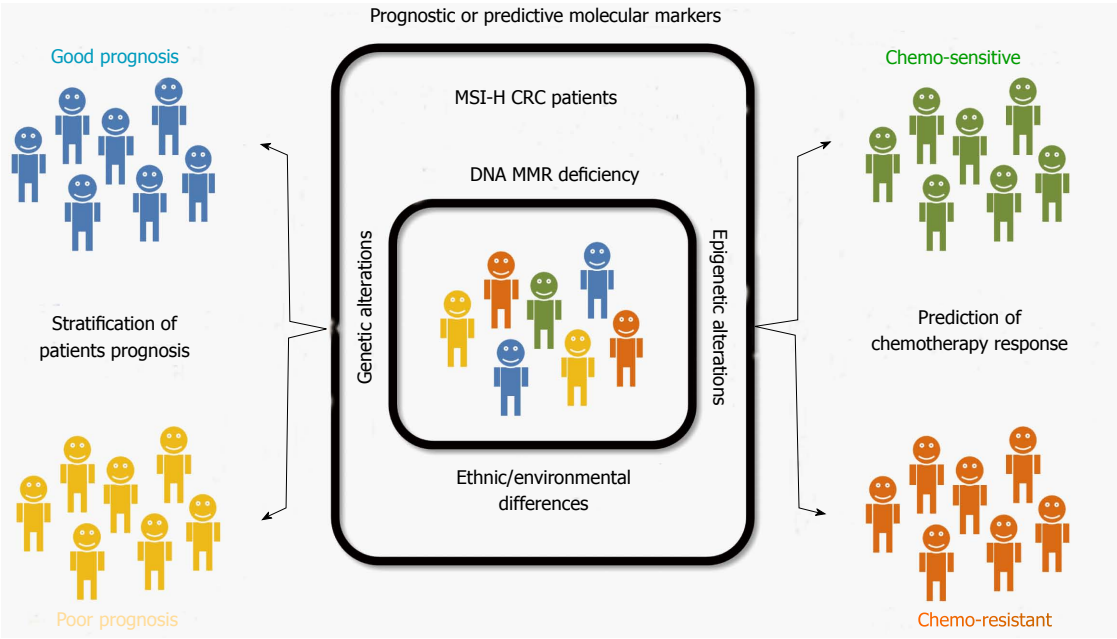

Figure 1 Graphical summary of this review, consisting of a conceptual model for prognostic heterogeneity based on the molecular heterogeneity of microsatellite-unstable colorectal cancers. CRC: Colorectal cancer; MSI-H: Microsatellite instability-high; MMR: Mismatch repair.

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 *BRAF* 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[92,93].

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.

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