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Association between Recurrent Metastasis from Stage II and III Primary Colorectal Tumors and Moderate Microsatellite Instability

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Abstract

Colorectal cancer (CRC) cells frequently have low levels of microsatellite instability (MSI-L) and elevated microsatellite alterations at tetranucleotide repeats (EMAST), but little is known about the clinicopathological significance of these features. We observed that patients with stage II or III CRC with MSI-L and/or EMAST had a shorter times of recurrence-free survival than patients with high levels of MSI (MSI-H) ($P=.0084$) or with highly stable microsatellites (H-MSS) ($P=.0415$), based on Kaplan-Meier analysis. MSI-L and/or EMAST were independent predictors of recurrent distant metastasis from primary stage II or III colorectal tumors (Cox proportional hazard analysis hazard ratio, 1.83; 95% confidence interval, 1.06–3.15; $P=.0301$).

Keywords

colorectal cancer; genetic instability; predictive factor; distant metastasis

Microsatellite assays using markers with only mononucleotide repeats clearly define microsatellite unstable, mismatch repair (MMR)-deficient CRC with high accuracy^{1, 2} (Supplementary Table 1). Using assays that include markers with mono- and di-nucleotide

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repeats such as the NCI reference panel, a small percentage of CRCs exhibiting low levels of MSI at dinucleotide repeat markers (MSI-L) are detected along with MSI-H, MMR-deficient CRCs and microsatellite stable (MSS) CRCs³ (Supplementary Table 1). While there are clear differences in clinicopathological behaviors and molecular profiles between MSI-H and MSI-L CRCs, and between MSI-H and MSS CRCs,^{3, 4, 5, 6} the distinction between MSI-L and MSS CRCs has been controversial.^{3, 7, 8} Previously we have shown that when EMAST markers were included in the MSI assay in addition to the NCI markers, all of the MSI-H CRCs exhibited high levels of instability in the EMAST markers whereas most of the MSI-L, and about half of the MSS CRCs exhibited instability in some of the EMAST markers.^{9, 10} Furthermore, MSI-L and EMAST in sporadic CRC appear to be a consequences of loss of MSH3 activity⁹ (Supplementary Table 1) and EMAST is commonly seen in metastases from rectal tumors.¹¹ These observations led us to speculate that MSI-L and EMAST CRCs, termed “moderate MSI” (or MSI-M) in this study, may belong to a clinicopathological group that is distinct from CRC with MSI-H and highly stable microsatellite CRCs (H-MSS), which is CRC that does not exhibit MSI at any NCI or EMAST markers.

To test this hypothesis, we determined the MSI status of 167 consecutive cases of primary CRC and matching normal tissues collected during a follow-up period of at least 5 years (Supplementary Methods). MSI was examined using 14 markers: 7 standard NCI and 7 EMAST markers (Supplementary Methods). Tumors were categorized according to MSI status using 3 different schemes, each with 3 categories. The first divided the CRCs into: MSI-H (instability at >3/7 NCI markers), MSI-L (instability at 1 or 2 of 7 NCI markers) and MSS (no instability at any NCI marker). The second grouping was: MSI-H, EMAST (non-MSI-H tumors with instability at >1/7 EMAST markers), and non-EMAST (non-MSI-H tumors without instability at any of the 7 EMAST markers). The third scheme sorted by: MSI-H, MSI-M (MSI-L and/or EMAST tumors), and H-MSS tumors without instability at any of the 7 NCI or 7 EMAST markers (Supplementary Table 1). According to our definitions, we identified 10 MSI-H, 24 MSI-L, and 133 MSS CRC in the first scheme; 10 MSI-H, 66 EMAST, and 91 non-EMAST CRC the second scheme; and 10 MSI-H, 80 MSI-M and 77 H-MSS in the third (Supplementary Fig. 1, Supplementary Table 2).

We first determined whether any clinicopathological characteristic (i.e., age, sex, tumor grade, location, stage and presence or absence of adjuvant chemotherapy) was associated with the CRC groups defined in each scheme. As anticipated, proximal location was significantly associated with MSI-H when compared to any non-MSI-H tumor type (Supplementary Tables 3 and 4). Distant recurrent metastasis occurred in 62/167 CRCs. We estimated recurrence-free survival (RFS) among 133 cases of stage II or III CRCs using the Kaplan-Meier method, and found a significant difference in RFS between MSI-H and MSI-L ($P=0.019$), and between MSI-H and MSS ($P=0.018$), but not between MSI-L and MSS ($P=0.578$) in the first categorization (Fig. 1A). Similarly, a significant difference was detected in RFS between MSI-H and EMAST ($P=0.009$), and between MSI-H and non-EMAST ($P=0.029$), but not between EMAST and non-EMAST ($P=0.155$) in the second scheme (Fig. 1B). However, the MSI-H, MSI-M and H-MSS patients in the third categorization showed significantly different RFS from one another (Fig 1C). Distant metastasis was more likely to occur in MSI-M tumors than in H-MSS ($P=0.0415$). Only when MSI-L and EMAST were included with MSI-M could they be recognized as a high-risk group among the non-MSI-H patients. Furthermore, when compared to H-MSS CRCs by multivariate Cox proportional hazard analysis, MSI-M is an independent predictor of recurrent distant metastasis from stage II/III primary CRCs (Table 1, hazard ratio: 1.83, 95% CI: 1.06–3.15, $P=0.03$). We then estimated the RFS for stage II and III CRCs separately. Although a significant difference in RFS was not detected between MSI-M and H-MSS in stage III CRC ($P=0.375$, 5 year RFS: 28.1% v.s. 44.9%), a strong trend for a

shorter RFS was observed in stage II MSI-M compared with stage II H-MSS CRC ($P=0.061$, 5 year RFS: 58.7% v.s. 81.1%). Further study using a larger cohort is warranted to confirm that MSI-M is associated with high-risk stage II CRC.

Consistent with other studies,^{3, 4, 5, 12} we showed that MSI-H is associated with proximal location and the lowest risk for recurrent distant metastasis. Importantly, MSI-M is an independent predicative marker of recurrent distant metastasis in stage II/III CRC. Although all MSI-H and a large portion of MSI-M tumors exhibited EMAST marker instability, the former is due to a lack of MLH1 or MSH2, leading to instability at any microsatellite target with a mono-, di- tri- or tetra-nucleotide repeat including EMAST, whereas the latter is most likely due to a lack of MSH3, leading to instability at microsatellite loci with di- or tetra-nucleotide repeats, including EMAST markers⁹ (Supplementary Table 1). Loss of MSH3 causes MSI-M,⁹ and MSH3 is repressed by hypoxia.¹³ Hypoxia is permissive of genetic instability and promotes the metastatic potential of primary tumors.^{14, 15} We speculate that hypoxia is the mechanistic link between MSI-M and recurrent metastasis.

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Abbreviations

CRC	colorectal cancer
EMAST	elevated microsatellite alterations at selected tetranucleotide repeats
MSI-L	low levels of microsatellite instability
MSI-H	high levels of microsatellite instability
MSI-M	moderate levels of microsatellite instability
H-MSS	highly stable microsatellites.

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Fig. 1. Kaplan-Meier analysis for recurrence-free survival in patients with stage II/III primary CRC

Recurrence-free survival rates in stage II/III CRC; (A) subdivided by MSI-H, MSI-L and MSS. MSI-H vs MSI-L ($P=0.019$), MSI-H vs MSS ($P=0.018$), MSI-L vs MSS ($P=0.578$); (B) subdivided by MSI-H, EMAST and non-EMAST. MSI-H vs EMAST ($P=0.009$), MSI-H vs non-EMAST ($P=0.029$), EMAST vs non-EMAST ($P=0.155$) (C) subdivided by MSI-H, MSI-M and H-MSS. MSI-H vs MSI-M ($P=0.008$), MSI-H vs H-MSS ($P=0.036$), MSI-M vs H-MSS ($P=0.0412$).

#: not significant

*: $P < 0.05$. P values were determined by log-rank test.

Table 1

Multivariate analysis for recurrent distant metastasis of primary CRC

Factors	Hazard Ratio	95%CI	P values
MSI-M vs H-MSS	1.83	1.06–3.15	0.03
Age <62 vs >62	0.91	0.52–1.56	0.73
Male vs female	1.04	0.60–1.80	0.87
Grade ^a G2+G3 vs G1	1.77	1.00–3.12	0.051
Location ^b distal vs proximal	1.47	0.64–3.35	0.35
Chemotherapy ^c yes vs no	1.7	0.60–4.76	0.31
Stage III vs II	2.17	1.16–4.05	0.015

^a G1: well differentiated, G2: moderately differentiated, G3: poorly differentiated.

^b Proximal includes cecum, ascending and transverse colon. Distal includes descending colon, sigmoid colon and rectum.

^c Some patients (stage II and III) received 5-FU-based adjuvant chemotherapy. Others did not.