

# Signet ring cell carcinoma of the colorectum: correlations between microsatellite instability, clinicopathologic features and survival

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Colorectal cancer with microsatellite instability has a characteristic clinicopathologic profile, featuring right-sided, lymphocyte-rich tumors with a better prognosis than microsatellite stable (MSS) carcinoma. Mucinous and signet ring cell carcinomas are both over-represented among microsatellite instability-high cancers. The clinicopathologic features of mucinous microsatellite instability-high cancer parallel those of the overall microsatellite instability-high set, but it is not known whether the same is true for signet ring cell carcinoma, particularly given the fact that signet ring histology is a well-documented adverse prognostic factor. We recorded age, sex, tumor size, site, grade, stage, histologic pattern, growth pattern, Crohn-like reaction, vascular invasion and tumor-infiltrating lymphocytes in 72 resected signet ring cell carcinomas of the colorectum. Microsatellite instability was determined by a combination of polymerase chain reaction and immunohistochemical stains for hMLH1, hMSH2 and hMSH6. Tumors with instability at >30% of informative markers and/or loss of hMLH1 or hMSH2 expression were designated microsatellite instability-high; all others were classified as MSS. A total of 22 (31%) signet ring cell carcinomas were microsatellite instability-high. Compared to MSS signet ring cell carcinoma, they were more likely to be right-sided (81 vs 45%,  $P=0.005$ ) and to affect older patients (68 vs 26%,  $P=0.0007$ ) of female sex (59 vs 28%,  $P=0.03$ ). Crohn-like reaction (45 vs 16%,  $P=0.02$ ) and high tumor infiltrating lymphocyte counts (32 vs 8%,  $P=0.03$ ) were more common in the microsatellite instability-high setting. There was no significant difference in 5-year survival in microsatellite instability-high vs MSS patients (41 vs 34%,  $P=0.3$ ). In conclusion, approximately one-third of signet ring carcinomas of the colorectum are microsatellite instability-high. Microsatellite instability-high signet ring carcinomas share clinicopathologic features with other microsatellite instability-high cancers: older age group, female preponderance, right-sided location, Crohn-like reaction and numerous tumor-infiltrating lymphocytes. Microsatellite instability status does not appear to be a significant predictor of survival in signet ring cell carcinoma of the colorectum.

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The phenomenon of microsatellite instability is observed in all colorectal cancers in the setting of hereditary nonpolyposis colorectal cancer as well as 15–20% of sporadic colorectal cancers.<sup>1–3</sup> Sporadic carcinomas with high-level microsatellite instability (MSI-H) tend to arise in the elderly, affect female population more than males, and involve the right side of the colon.<sup>1–7</sup> Histologically, medullary, mucinous and signet ring patterns are more common than in microsatellite stable (MSS) cancers.<sup>4–9</sup> Many tumor-infiltrating lymphocytes

and a peritumoral Crohn-like infiltrate are also characteristic.<sup>4–9</sup> Colon cancer with MSI-H has a better prognosis than other colon cancers; the difference is partly dependent on stage (microsatellite instability-high tumors tend not to produce metastatic disease), but has been found to be independent of stage in some studies.<sup>10–16</sup>

Signet ring cell carcinoma is a rare subtype of colorectal cancer, accounting for 0.1–2.4% of all colorectal malignancy.<sup>17–20</sup> By definition, more than 50% of tumor cells must have signet ring cell morphology.<sup>21</sup> Many studies have demonstrated the aggressive behavior of signet ring cell carcinoma.<sup>17–20,22–25</sup> The American Joint Committee on Cancer (AJCC) and the College of American Pathologists accept signet ring histology to be an independent adverse prognostic factor.<sup>26,27</sup>

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This gives rise to a paradoxical situation: microsatellite instability-high colon cancers have favorable prognosis, but can often show signet ring morphology, a well-documented adverse prognostic factor. It is not known whether the signet ring cell carcinoma with MSI-H shares the favorable outcome associated with other colorectal cancer with microsatellite instability. The College of American Pathologists has recommended that 'tumor type should be correlated with outcome after adjustment for microsatellite instability status in statistically robust studies with multivariate analysis, in order to definitively determine its prognostic significance'.<sup>27</sup> We studied associations between microsatellite instability status, clinicopathologic features and survival in a series of 72 resected colorectal signet ring cell carcinomas.

## Methods

### Patients

A consecutive series of 62 patients who underwent resection for signet ring cell carcinomas was identified from the pathology files at Mayo Clinic Rochester between 1985 and 2000. In total, 10 additional patients were identified from the North Central Cancer Treatment Group. Primary signet ring cell carcinoma was defined as a colonic neoplasm in which more than 50% of the tumor was composed of signet ring cells. Clinical parameters, including age, sex, date of surgery, tumor size, site and stage were obtained from the pathology reports. Tumors in the cecum, ascending colon and transverse colon were classified as right-sided and those in the descending colon, sigmoid and rectum were left-sided. Clinical charts were reviewed to obtain information about distant metastases, adjuvant therapy and the presence of underlying conditions such as inflammatory bowel disease, familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer. Survival data were obtained from the Mayo tumor registry; clinical follow-up ranged from 1 to 188 months (mean 37.4). The study was approved by the Institutional Review Board.

### Pathologic Features

Hematoxylin and eosin (H&E)-stained slides were examined by two pathologists who did not know clinical, immunohistochemical or microsatellite instability results. The following features were evaluated:

(1) *Signet ring and mixed growth patterns*: By definition, all tumors had more than 50% signet ring cells (signet ring cells were required to have an intracytoplasmic mucin-filled vacuole caus-

ing lateral displacement of the nucleus). The presence of other histologic subtypes (conventional adenocarcinoma, mucinous carcinoma, medullary carcinoma) was noted.

(2) *Tumor-infiltrating lymphocytes*: The tumor sections were scanned at low power to ascertain areas with the highest number of tumor-infiltrating lymphocytes. The average number of lymphocytes within the tumor epithelium per high field in these areas was determined by counting 10 high-power fields.

(3) *Crohn-like lymphoid infiltrate*: This refers to the presence of lymphoid aggregates at the advancing edge of the tumor. This feature was considered to be present if two or more lymphoid aggregates were seen per tissue section.

(4) *Growth pattern*: The growth pattern was recorded as pushing when the advancing front of the tumor was sharply defined, and infiltrating if there was irregular infiltration of tumor cells at the tumor-stroma interface.

(5) Vascular invasion (evaluated without the use of special stains).

(6) Depth of invasion (mucosa, submucosa, muscularis propria, subserosal fat, serosa)

(7) Lymph node metastasis.

(8) Depth of tumor invasion, lymph node status and clinical information were used to assign tumor stage using the TNM system described in the AJCC Cancer Staging Manual.<sup>28</sup>

### Immunohistochemistry

Immunohistochemistry was performed manually by using mouse monoclonal antibodies against mismatch repair enzymes hMLH1, hMSH2 and hMSH6. Formalin-fixed paraffin-embedded sections were rehydrated using xylene and graded alcohol washes. After blocking the endogenous peroxidase, heat-induced antigen retrieval was performed with EDTA buffer, pH 8.0 in a steamer for 5 min, and the sections were incubated with primary antibody (hMLH1 1:200, Pharmingen, hMSH2 1:50, Oncogene Sciences and hMSH6 1:500, Transduction Laboratories) for 60 min. Following washing with PBS, sections were incubated with the biotinylated goat anti-mouse secondary antibody (1:100) for 30 min. Sections were incubated with avidin-biotin complex (ABC Elite Kit, Vector Laboratories, Burlingame, CA, USA) for 20 min, developed with diacetyl benzidine for 5–10 min and counterstained with hematoxylin. The adjacent normal colonic mucosa and stromal cells with intact mismatch repair function served as the internal control. Immunohistochemistry results were classified as positive or negative. Any nuclear staining was considered a positive result. Tumors with no nuclear staining for hMLH1, hMLH2 and/or hMLH6 were classified as negative.

## Microsatellite Analysis

Paired normal and tumor DNA were analyzed for microsatellite instability. A desired area of the normal and tumor tissue was selected on H&E-stained slides. The selected tissue was scraped from the corresponding unstained unbaked 10- $\mu$ m sections. DNA was extracted using the Qiamp Tissue Kit (Qiagen, Inc., Santa Clarita, CA, USA) using previously established procedure. The extracted DNA served as a template for polymerase chain reaction using 10 microsatellite markers: Bat 26, D17S250, D5S346, ACTC, D18S55, Bat 40, D10S197, Bat 34c4, MycL and Bat 25. Tumors with microsatellite instability at >30% of the informative loci were classified as microsatellite instability-high. Tumors with no instability or instability at <30% of markers tested were designated MSS.

## Statistical Analysis

The relationship between variables was tested by  $\chi^2$  and Fisher's exact tests. In the survival analysis, the starting point for the survival time was the date of surgery. Survival curves were calculated using the Kaplan–Meier method and statistical significance between curves was tested by the log-rank test. Univariate analysis was performed using Cox proportional hazards model; the hazard ratio and its 95% confidence intervals were assessed for each factor. *P*-values of <0.05 were considered statistically significant.

## Results

In total, 22 (31%) signet ring cell carcinomas had MSI-H. One microsatellite instability-high cancer showed intact immunohistochemical staining for all three mismatch repair proteins tested; the other 21 lacked staining for hMLH1. Immunohistochemical stains for hMSH2 and hMSH6 showed intact expression of those proteins in all cases. There was no loss of staining in any MSS tumor. Thus, immunohistochemical staining was 95% sensitive and 100% specific for MSI-H in these tumors. The mean age for all patients was 61.6 years (range 26–86 years); nine (13%) were less than 40 years old. The location of the tumor was right colon in 39 patients (56%), descending or sigmoid colon in 18 (26%) and rectum in 13 (18%) cases. All of the rectal tumors were MSS. Adjuvant therapy was administered in 39/66 (59%) patients (no information was available for six patients). There was no significant difference in 5-year survival in patients with or without adjuvant therapy (41 vs 37%; *P*=0.3). Five (7%) patients had underlying inflammatory bowel disease. No patient had a recorded diagnosis of familial adenomatous polyposis or hereditary nonpolyposis colorectal cancer.

As shown in Table 1, microsatellite instability-high signet ring cell carcinomas were more likely to occur in older patients, female cases, and in the right colon. Crohn-like reaction and high numbers of tumor-infiltrating lymphocytes were more common in the microsatellite instability-high setting. There was no correlation between microsatellite instability status and tumor size, grade, growth pattern or vascular invasion.

The relationship of clinical and pathologic variables with survival is shown in Table 2. The 5-year survival was 41% (95% CI: 23–73%) in the

**Table 1** Correlation of microsatellite instability with clinico-pathologic features and survival

|                            | MSI-high | MSS      | P-value |
|----------------------------|----------|----------|---------|
| <i>Age (years)</i>         |          |          |         |
| <70                        | 7 (32)   | 37 (74)  | 0.0007  |
| ≥70                        | 15 (68)  | 13 (26)  |         |
| <i>Gender</i>              |          |          |         |
| Female                     | 13 (59)  | 14 (28)  | 0.03    |
| Male                       | 9 (41)   | 36 (72)  |         |
| <i>Site</i>                |          |          |         |
| Right                      | 17 (81)  | 22 (45)  | 0.005   |
| Left                       | 4 (19)   | 27 (55)  |         |
| <i>Size (cm)</i>           |          |          |         |
| <5                         | 7 (32)   | 13 (26)  | 0.6     |
| ≥5                         | 15 (68)  | 37 (74)  |         |
| <i>Growth</i>              |          |          |         |
| Expanding                  | 0        | 0        | —       |
| Infiltrating               | 22 (100) | 50 (100) |         |
| <i>Histologic type</i>     |          |          |         |
| Pure SRC                   | 12 (63)  | 33 (77)  | 0.4     |
| SRC+other subtypes         | 7 (37)   | 10 (23)  |         |
| <i>Vascular invasion</i>   |          |          |         |
| Present                    | 8 (36)   | 22 (44)  | 0.6     |
| Absent                     | 14 (64)  | 28 (56)  |         |
| <i>Extracellular mucin</i> |          |          |         |
| <70%                       | 10 (45)  | 23 (46)  | 1       |
| ≥70%                       | 12 (55)  | 27 (54)  |         |
| <i>TIL</i>                 |          |          |         |
| <2 per HPF                 | 15 (68)  | 46 (92)  | 0.027   |
| ≥2 per HPF                 | 7 (32)   | 4 (8)    |         |
| <i>Crohn-like reaction</i> |          |          |         |
| Absent                     | 12 (55)  | 42 (84)  | 0.028   |
| Present                    | 10 (45)  | 8 (16)   |         |
| <i>Stage</i>               |          |          |         |
| I                          | 0        | 1 (2)    | 0.3     |
| II                         | 8 (36)   | 8 (16)   |         |
| III                        | 11 (50)  | 31 (62)  |         |
| IV                         | 3 (14)   | 10 (20)  |         |
| <i>Survival</i>            |          |          |         |
| <5 years                   | 13 (59)  | 33 (66)  | 0.3     |
| ≥5 years                   | 9 (41)   | 17 (34)  |         |

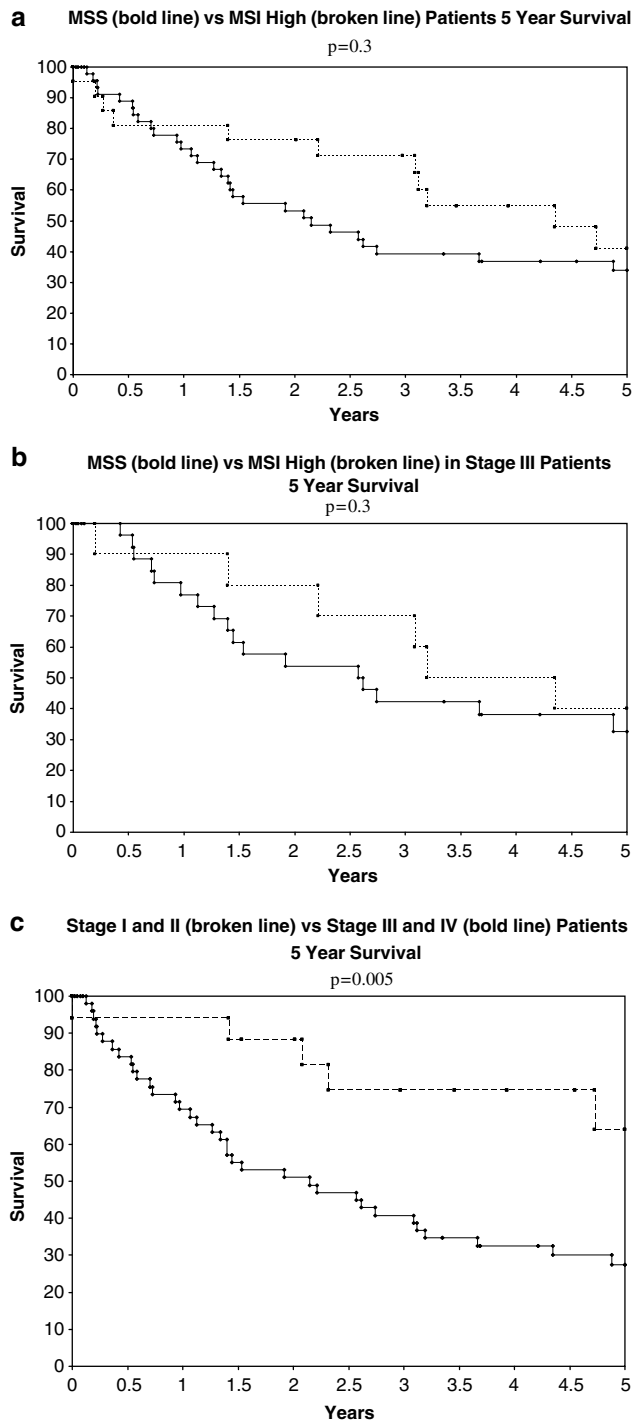
**Table 2** Correlation of microsatellite instability and clinico-pathologic features with survival

|                            | 5-year survival %<br>(95% confidence intervals) | P-value |
|----------------------------|---|---------|
| <b>MSI status</b>          |   |         |
| MSI-H                      | 34 (22–52)                                      | 0.3     |
| MSS                        | 41 (23–73)                                      |         |
| <b>Stage</b>               |   |         |
| A, B                       | 68 (46–100)                                     | 0.005   |
| C, D                       | 28 (17–44)                                      |         |
| <b>Age (years)</b>         |   |         |
| <70                        | 33 (20–53)                                      | 0.9     |
| ≥70                        | 42 (26–68)                                      |         |
| <b>Sex</b>                 |   |         |
| Female                     | 36 (20–63)                                      | 0.7     |
| Male                       | 37 (25–56)                                      |         |
| <b>Site</b>                |   |         |
| Right                      | 33 (20–54)                                      | 0.6     |
| Left                       | 47 (27–80)                                      |         |
| <b>Vascular invasion</b>   |   |         |
| Present                    | 42 (29–62)                                      | 0.4     |
| Absent                     | 16 (5–52)                                       |         |
| <b>Tumor lymphocytes</b>   |   |         |
| <2 per HPF                 | 36 (26–52)                                      | 0.3     |
| ≥2 per HPF                 | 41 (29–81)                                      |         |
| <b>Crohn-like reaction</b> |   |         |
| Absent                     | 33 (21–50)                                      | 0.1     |
| Present                    | 47 (27–79)                                      |         |

microsatellite instability-high group and 34% (95% CI: 22–52%) in the MSS group with  $P = 0.3$  (Figure 1a). Survival was not significantly different for microsatellite instability-high vs MSS even when stratified by stage (Figure 1b). Age, gender, site and size of tumor, tumor-infiltrating lymphocytes, Crohn-like infiltrate, growth pattern and vascular invasion all showed no correlation with 5-year survival. The 5-year survival (including both microsatellite instability-high and MSS cases) for low-stage patients (stages I and II) was 68% compared to 28% for high stage (stages III and IV) with  $P = 0.005$  (Figure 1c).

## Discussion

MSI-H is an independent predictor of relatively favorable outcome<sup>10–16</sup> and decreased likelihood of metastasis<sup>13</sup> in sporadic colorectal carcinoma. In most of these reports, histologic subtypes are not discussed. Signet ring cell carcinoma finds mention only in the study by Gryfe *et al*<sup>13</sup> in which 19 of the 607 colorectal patients had signet ring morphology. The correlation of MSI status with survival in signet ring cell carcinoma has not been examined, so that it



**Figure 1** Kaplan–Meier curves demonstrating 5-year survival in signet ring carcinoma: (a) MSI vs MSS, all patients, (b) MSI vs MSS in stage III patients, and (c) low stage vs high stage disease (including both microsatellite instability-high and MSS cases).

is not known whether the favorable prognosis for microsatellite instability-high tumors as a group encompasses the signet ring cell subtype.

We found that approximately one-third (31%) of signet ring cell carcinomas of the colorectum are microsatellite instability-high. The overall 5-year

survival for our patients was 38%; in other large series concerning this histologic subtype, 5-year survival has ranged from 9 to 37%.<sup>17–20,22,29</sup> While the 5-year survival for our patients with microsatellite instability-high cancers was marginally better than those without MSI-H (41 vs 34%), the difference was not statistically significant ( $P=0.3$ ). Statistically significant improved survival was not observed even when the two groups were stratified according to stage.

Why does the adverse influence of signet ring cell morphology outweigh the beneficial influence of microsatellite instability? Probably because classifying tumors as either microsatellite instability-high or MSS is an oversimplification. Whitehall *et al*<sup>30</sup> have identified a subset of MSS carcinomas with clinicopathologic features of microsatellite instability-high cancer; this subset develops as a result of hypermethylation at loci other than hMLH1. Goel *et al*<sup>31</sup> suggest that colon cancer can be divided into four subsets: those with microsatellite instability but without multiple loss of heterozygosity events, those with loss of heterozygosity but not microsatellite instability, those with neither loss of heterozygosity nor microsatellite instability, and those with both loss of heterozygosity and microsatellite instability. Colorectal cancers with high level loss of heterozygosity behave more aggressively than those with fewer loci involved.<sup>32</sup> One recent study found high level loss of heterozygosity in signet ring cell carcinoma, including those with microsatellite instability.<sup>33</sup> Thus, even though our patients with microsatellite instability-high signet ring cell carcinoma had clinicopathologic features associated with the microsatellite instability-high phenotype (proximal colon, increased age, female sex, prominent host lymphoid response), it may be that signet ring morphology is a marker for additional genetic abnormalities associated with poor outcome. Our series differs slightly from other studies of signet ring cell carcinoma. Young age of onset has been a feature of several reports, with 30–63% of patients less than 40 years old at presentation.<sup>18,19,29</sup> In contrast, only 12.5% of our patients were less than 40 years old, a result in agreement with another recent study that reported a figure of 15%.<sup>20</sup> The rectum has been disproportionately involved in some studies, although figures have varied from 20 to 40%.<sup>17–20</sup> Our results fall near the lower end of that range. The outcome for these patients was similar to patients with signet ring cell carcinomas at other sites in the colon.

In summary, approximately one-third of signet ring cell carcinomas of the colorectum have MSI-H. Microsatellite instability-high signet ring carcinomas have clinicopathologic features similar to those of other microsatellite instability-high colorectal cancers. Microsatellite instability status does not appear to be a significant predictor of survival in signet ring cell carcinoma of the colorectum. In our study, tumor stage is the only variable that correlates

with outcome (5-year survival 68% for Stage I and II combined, vs 28% for Stage III and IV combined;  $P=0.005$ ).

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