detected early through screening such as breast and colon cancers. In these papers by Gomez and Liu, the authors’ discussions of cancer etiology are limited and can be more completely understood as the immigration patterns of these populations are investigated.

References


Notes

The authors declare no conflicts of interest.

Affiliations of authors: Public Health Institute, Cancer Registry of Greater California, Sacramento, CA (PKM); Fresno Medical Education and Research Program, Department of Medicine, University of California, San Francisco, CA University of California at San Francisco, Fresno, CA (PKM).

BRAF Mutation and Microsatellite Instability Status in Colonic and Rectal Carcinoma: Context Really Does Matter

Stanley R. Hamilton

Correspondence to: Stanley R. Hamilton, MD, University of Texas MD Anderson Cancer Center, Division of Pathology and Laboratory Medicine, 1515 Holcombe Blvd, Box 85, G1.3540, Houston, TX 77030 (e-mail: shamilton@mdanderson.org)

The molecular characteristics of colorectal cancer (CRC) have been studied extensively since the 1980s, but translation of the remarkable increase in genomic knowledge into clinically used biomarkers has been distressingly slow. The Cancer Genome Atlas for CRC was published in mid-2012 (1), and molecular and pathologic findings including genetic and epigenetic abnormalities have now been incorporated into classification systems that have been reported to have implications for the clinical management of patients (2–4). Numerous individual molecular biomarkers with potential applications have been published, but few have achieved levels and breadth of evidence to become standard of care. Difficulty in convincing payers of the value of biomarkers and fiscal constraints have impeded adequate reimbursement for testing and disincentsitized their clinical use.

In this issue of the Journal, Lochhead and colleagues (5) provide important additional evidence supporting the routine clinical use in CRC patients of two extensively investigated molecular alterations: microsatellite instability and BRAF mutation. Both of these characteristics of CRC are in use as biomarkers (6), but they have been uncommonly addressed together in the four microsatellite instability (MSI)/BRAF subgroups for clinical usage in prognostication.

High levels of MSI (MSI-H) occur in about 15% of CRC, and the presence of this feature is a hallmark of Lynch syndrome (hereditary nonpolyposis colorectal cancer syndrome). Although most MSI-H CRC are sporadic because of acquired hypermethylation of the MLH1 mismatch repair gene promoter region, germline mutation in one of several mismatch repair genes, most frequently MLH1 or MSH2, results in MSI-H tumors in Lynch syndrome patients. At least three professional organizations (7–9) have issued recommendations for routine testing for MSI status in CRC to identify tumors in patients who should be evaluated further for Lynch syndrome because of the implications for family members as well as the affected patient with an MSI-H tumor. In addition, abundant evidence supports MSI-H as a favorable biomarker for improved stage-specific survival, and testing for MSI status has therefore demonstrated value as a prognostic marker, also contributing to its frequent routine evaluation in CRC.

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Mutation of the BRAF proto-oncogene at codon 600 is infrequent in CRC, occurring in single-digit percentages in numerous publications. This low frequency has made study of the clinical implications difficult because of small sample sizes of CRC patients whose tumor has the mutation, but the associations with MSI-H and CpG island methylator phenotype (CIMP) have been well established. BRAF mutation at codon 600, usually the V600E mutation, is well known as an adverse prognostic indicator, but the key first recognition of the importance of the non–MSI-H context was by Samowitz et al. in 2005 (10), subsequently confirmed by other investigators as cited by Lochhead et al. (5). Mutation of the BRAF gene was also found to be extremely rare in patients with Lynch syndrome (6). These two observations have resulted in frequent routine clinical testing of CRC for BRAF mutation.

The study by Lochhead et al. (5) is one in a series of molecular investigations of CRC in subjects in the Nurses’ Health Study and Health Professionals Follow-up Study. This treasure trove of specimens with clinical annotation has been characterized for a wide variety of molecular alterations to provide evidence for clinically useful biomarkers. The database provided 1253 tumors for this report (5) and yielded relatively large sample sizes of the two infrequent MSI/BRAF subgroups of CRC that are of particular interest (ie, those with BRAF V600 mutation with and without MSI-H [n = 101 and 81 tumors, respectively]). The results of the study are highlighted in Table 1.

The tumors analyzed for the study appear to be a relatively representative population of CRC, although the frequency of BRAF V600 mutation is high (15%), as is the frequency of known stage I tumors (26%). In addition, women outnumber men, although this could be expected from the case ascertainment that included the Nurses’ Health Study. Unfortunately, follow-up treatment information was not available in the database, and the results of therapy could have impacted survival, which is a major focus of the study. The characteristics of the CRC in the study do have expected associations: 55% of the BRAF-mutated CRC had MSI-H, and poorly differentiated CRC was most frequent in the MSI-H groups (40% with and 23% without BRAF V600 mutation, respectively), as was CIMP-high (99% and 51%, respectively). The expected nearly exclusive relationship between BRAF and KRAS mutations was found, with only six tumors (0.5%) having both mutations.

The adverse prognosis of non–MSI-H CRC with BRAF V600 mutation as reported in previous studies was confirmed in the survival analyses that include stage stratification. The 5-year CRC-specific survival rate for such tumors across all stages was only 46%, as contrasted with 65% for non–MSI-H tumors without identified BRAF mutation, 73% for MSI-H tumors with BRAF V600 mutation, and 79% for MSI-H tumors with no identified BRAF mutation (Table 1). Stage-specific rates were not presented, probably because of the small sample sizes of the MSI/BRAF subgroups. The CRC-specific and overall 5-year mortality hazard ratios for the non–MSI-H tumors with BRAF mutation were a modest 1.60 and 1.36, respectively, as compared with non–MSI-H CRC with no identified BRAF mutation (Table 1). The corresponding CRC-specific and overall 5-year mortality hazard ratios in the MSI-H tumors were 0.48 with BRAF mutation and 0.25 without and 0.84 with BRAF mutation and 0.58 without, respectively. There was, however, no statistically significant evidence for a differential prognostic role of BRAF mutation in the MSI-H subgroups (Pinteraction > .50). Poor differentiation is often used as an adverse prognostic finding in CRC (11–13), but the MSI-H tumors that have the highest frequency of poorly differentiated tumors (40% with and 23% without BRAF mutation) had the best survival rates in the study, providing another example of the importance of context.

This high-quality and important study of Lochhead et al. (5) helps to cement the concept that the context of BRAF mutation in CRC really does matter. It is clear from this study that MSI status must be evaluated and included in the data analysis of any study of CRC that addresses BRAF mutation, as MSI-H overrides BRAF V600 mutation in assessing prognosis. Much does remain to be learned, however. The explanation of the small subset of patients who have poor outcome from their MSI-H CRC is not evident.

### Table 1. Summary of selected findings in Lochhead et al. (5)*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All cases</th>
<th>BRAF V600 mutation, MSI-H</th>
<th>BRAF V600 mutation, MSS/MSI-L</th>
<th>No BRAF V600 mutation, MSI-H</th>
<th>No BRAF V600 mutation, MSS/MSI-L</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>1253</td>
<td>101</td>
<td>81</td>
<td>92</td>
<td>979</td>
</tr>
<tr>
<td>Known stage I</td>
<td>26%</td>
<td>18%</td>
<td>18%</td>
<td>26%</td>
<td>28%</td>
</tr>
<tr>
<td>Known stage II</td>
<td>31%</td>
<td>58%</td>
<td>18%</td>
<td>55%</td>
<td>27%</td>
</tr>
<tr>
<td>Known stage III</td>
<td>28%</td>
<td>19%</td>
<td>27%</td>
<td>13%</td>
<td>31%</td>
</tr>
<tr>
<td>Known stage IV</td>
<td>15%</td>
<td>5%</td>
<td>37%</td>
<td>7%</td>
<td>15%</td>
</tr>
<tr>
<td>Poor differentiation</td>
<td>10%</td>
<td>40%</td>
<td>19%</td>
<td>23%</td>
<td>5%</td>
</tr>
<tr>
<td>CIMP-high</td>
<td>17%</td>
<td>99%</td>
<td>40%</td>
<td>51%</td>
<td>4%</td>
</tr>
<tr>
<td>5-yr CRC-specific survival</td>
<td>Not presented</td>
<td>73%</td>
<td>46%</td>
<td>79%</td>
<td>65%</td>
</tr>
<tr>
<td>Multivariable 5-year CRC-specific mortality hazard ratio (95% CI)</td>
<td>Not presented</td>
<td>0.48 (0.27 to 0.87)</td>
<td>1.60 (1.12 to 2.28)</td>
<td>0.25 (0.12 to 0.52)</td>
<td>1.00 (—)</td>
</tr>
<tr>
<td>P</td>
<td>―</td>
<td>.02</td>
<td>.009</td>
<td>&lt;.001</td>
<td>―</td>
</tr>
<tr>
<td>Multivariable overall 5-yr mortality hazard ratio (95% CI)</td>
<td>Not presented</td>
<td>0.84 (0.59 to 1.19)</td>
<td>1.36 (1.00 to 1.84)</td>
<td>0.58 (0.40 to 0.85)</td>
<td>1.00 (—)</td>
</tr>
<tr>
<td>P</td>
<td>―</td>
<td>.32</td>
<td>.052</td>
<td>.005</td>
<td>―</td>
</tr>
</tbody>
</table>

* MSI = microsatellite instability; H = high; L = low; MSS = microsatellite stable; CIMP = CpG island methylator phenotype; CRC = colorectal cancer; CI = confidence interval.
BRAF V600 mutation alone does not account for this unfortunate subset, as some patients with MSI-H and no evident BRAF mutation do succumb to their disease despite the good prognosis of the subgroup as a whole. The impact of mutations in sites in the BRAF gene other than codon 600 is unknown and will be difficult to determine because of their extremely low frequency. The mechanisms of the mutation process and the failure of repair that result in the altered nucleotide sequence of the BRAF gene are incompletely explained.

Most important, although this study confirms that BRAF V600 mutation clearly confers lower survival in non–MSI-H CRC and therefore serves as a prognostic marker in this subgroup of patients, actionability is unproven. Different therapeutic approaches to patients in the four MSI/BRAF subgroups remain to be clarified. Although no stage-specific analysis was done in this study (5), a previous study has shown that stage is associated with the survival rates in the MSI/BRAF-defined subgroups, with the expected lower rates in patients with more advanced stage (10).

The subgroup of non–MSI-H CRC with BRAF mutation that has the worst survival among the four MSI/BRAF subgroups is tempting for therapeutic intervention, but those patients with stage I disease likely have such good survival [100% in a small study (10)] that postoperative adjuvant chemotherapy appears unwarranted. Although postoperative adjuvant chemotherapy is not recommended routinely for stage II CRC (11–13), the non–MSI-H/BRAF–mutated subgroup has poor survival [about 20% at 5 years (10)] and hazard ratio approaching 3 (14) in two studies that have addressed that stage of disease. Patients with stage IV or recurrent non–MSI-H CRC with BRAF V600 mutation are of particular interest for combination therapy, including a BRAF inhibitor (15,16), and additional clinical studies are now needed to translate the MSI/BRAF prognostic data into improved patient outcomes in this small subset of CRC patients.

References

Affiliation of author: Division of Pathology and Laboratory Medicine, University of Texas MD Anderson Cancer Center, Houston, TX.