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Original contribution

Should the grading of colorectal adenocarcinoma include microsatellite instability status?☆,☆☆

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Summary Adenocarcinomas of the colon and rectum are graded using a 2-tiered system into histologic low-grade and high-grade tumors based on the proportion of gland formation. The current grading system does not apply to subtypes of carcinomas associated with a high frequency of microsatellite instability (MSI), such as mucinous and medullary carcinomas. We investigated the combined effect of histologic grade and MSI status on survival for 738 patients with colorectal carcinoma (48% female; mean age at diagnosis 68.2 years). The proportion of high-grade adenocarcinoma was 18%. MSI was observed in 59 adenocarcinomas (9%), with higher frequency in high-grade tumors compared with low-grade tumors (20% versus 6%; $P < .001$). Using Cox regression models, adjusting for sex and age at diagnosis and stratifying by the American Joint Committee on Cancer stage, microsatellite stable (MSS) high-grade tumors were associated with increased hazard of all-cause and colorectal cancer–specific mortality: hazard ratio 2.09 (95% confidence interval [CI], 1.58-2.77) and 2.54 (95% CI, 1.86-3.47), respectively, both $P < .001$. A new grading system separating adenocarcinoma into low grade (all histologic low grade and

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MSI high grade) and high grade (MSS histologic high grade) gave a lower Akaike information criterion value when compared with the current grading system and thus represented a better model fit to stratify patients according to survival. We found that patients with a high-grade adenocarcinoma had significantly shorter survival than patients with low-grade adenocarcinoma only if the tumor was MSS, suggesting that the grading of colorectal adenocarcinoma with high-grade histologic features should be made according to the MSI status of the tumor.

1. Introduction

Most colorectal carcinomas are adenocarcinomas of usual type (adenocarcinoma NOS, not otherwise specified). Since the first grading systems were established [1,2], pathologists have routinely included histologic grade in their reports of resected colorectal adenocarcinomas. Adenocarcinomas are graded into well-differentiated, moderately differentiated, or poorly differentiated tumors (grades 1, 2, and 3, respectively) depending on the proportion of gland formation in the least differentiated component of the tumor away from the invasive edge, according to the World Health Organization (WHO) criteria [3]. Despite low levels of agreement among pathologists on this subjective assessment [4,5], histologic grading has been shown to be an independent prognostic factor for colorectal carcinoma [6-9]. This is particularly true for the poorly differentiated subgroup that has been most consistently found to be associated with adverse clinical outcome. The WHO and the American Joint Committee on Cancer (AJCC) recommend a 2-tiered histologic grading system: low grade for well-differentiated and moderately differentiated adenocarcinomas (50%-100% gland formation) and high grade for poorly differentiated adenocarcinomas (0%-49% gland formation) [3,10].

Testing tumors for microsatellite instability (MSI) by immunohistochemistry for mismatch repair (MMR) proteins MLH1, MSH2, PMS2, and MSH6 and/or by molecular-based methods is routinely performed for patients diagnosed with colorectal carcinoma, primarily to screen for Lynch syndrome. Up to 15% of all colorectal carcinomas demonstrate MSI, more frequently secondary to acquired methylation of *MLH1* (sporadic cases) than caused by a germline mutation in an MMR gene (Lynch syndrome). MSI has been reported to be a strong positive prognostic factor by multiple independent studies [11-13]. Some histologic subtypes of colorectal carcinomas are more commonly observed in MSI tumors, including medullary carcinomas, mucinous adenocarcinomas, and signet ring cell carcinomas [14]. The adverse prognosis associated with the poor differentiation of most of these tumor subtypes contrasts with the positive prognosis associated with MSI. Consequently, the current WHO histologic grading does not apply to these subtypes of colorectal carcinoma. In addition, the WHO recommends that mucinous carcinomas should be graded according to their MSI status, regardless of their morphological appearance [3]. Such an MSI-based grading principle could potentially be applied to colorectal adenocarcinomas of usual type to more effectively stratify patients by prognosis. To test this hypothesis, we investigated the survival of a large series of patients diagnosed with colorectal adenocarcinoma with respect to histologic grade and MSI status.

2. Materials and methods

2.1. Study sample

Incident colorectal carcinomas were identified from participants enrolled in the Melbourne Collaborative Cohort Study (MCCS), a prospective cohort study of 41 514 people (17 045 males and 24 469 females) recruited between 1990 and 1994 [15]. Participants were aged 27 to 75 years with almost all aged between the ages 40 to 69 years at baseline. The study protocol was approved by the Cancer Council Victoria's Human Research Ethics Committee and the human research ethics committee of the Queensland Institute of Medical Research under protocol P799. Written informed consent was obtained from all study subjects for the investigators to review their medical records.

2.2. Data collection

Clinical data were collected from medical charts, colonoscopy reports, and pathology reports. Paraffin-embedded tissue blocks were collected from hospital pathology departments where the patient underwent colectomy. Tissue sections were cut for pathology reviews, immunohistochemistry, and DNA extraction. All surgically resected carcinomas underwent standardized review by 2 pathologists (Jeremy Jass and Christophe Rosty) to assess for a set of histologic features including histologic type (adenocarcinoma, mucinous carcinoma, others) and tumor grade. Adenocarcinoma of usual type is defined by a carcinoma of intestinal type forming glandular structures with variability in size and configuration and with frequent mucus and cellular debris in the lumen. In poorly differentiated adenocarcinomas, gland formations had to be present even if only focally. Following the 2010 WHO histologic grading system, adenocarcinomas were