The Role of Defective Mismatch Repair in Small Bowel Adenocarcinoma in Celiac Disease

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ABSTRACT

Celiac disease is associated with an increased risk of small bowel adenocarcinoma. The aims of this study were to investigate the molecular basis, assess outcomes, and identify clinicopathologic characteristics of small bowel adenocarcinoma in celiac disease. retrospective case control cohort study of all celiac disease patients treated at our institution for small bowel adenocarcinoma and matched control patients with sporadic small bowel adenocarcinoma from July 1960 to November 2002. Mismatch repair (MMR) status was assessed by testing tissue for microsatellite instability (MSI) and for hMLH1 and hMSH2 protein expression. Over a 40-year time period, 18 patients with small bowel adenocarcinoma and celiac disease were treated at the Mayo Clinic. One celiac disease patient was excluded. High-frequency MSI (MSI-H) was identified in 8 of 11 (73%) and 2 of 22 (9%) available small bowel adenocarcinoma specimens in the celiac disease and control groups, respectively. In the celiac disease group, MSI-H was associated with loss of hMLH1 and hMSH2 in 6 and 1 specimen, respectively. Loss of hMLH1 occurred in both control tumors. Stage was associated with celiac disease status (P = 0.018), and 78% of controls were stage III or IV compared with 47% of celiac disease patients. Overall, survival was better (P = 0.025) in the celiac disease group compared with stage-matched controls. Celiac disease patients with small bowel adenocarcinoma had a high incidence defective MMR (73%) compared with controls and had better survival compared with stage-matched controls. In addition, celiac disease patients presented more frequently with early-stage small bowel adenocarcinoma. The better survival and earlier presentation of small bowel adenocarcinoma in celiac disease appears to be biologically associated with defective MMR.

INTRODUCTION

Celiac disease is an inflammatory condition of the small bowel triggered by dietary gluten in genetically susceptible individuals. Patients with celiac disease are at greater risk than the general population for the development of malignant neoplasms (1–3). Although the most common neoplasm associated with celiac disease is intestinal lymphoma, celiac disease patients carry a 10 to 280-fold increased risk of small bowel adenocarcinoma compared with the general population (4, 5). Restriction of dietary gluten resolves the pathological changes in the small bowel mucosa and has been suggested to be protective in the development of malignancy (3); however, this has not been conclusively demonstrated (6, 7). Similarly, the mechanisms for the development of small bowel adenocarcinoma are largely unknown.

Similarities between adenocarcinoma of the small bowel and colorectal carcinoma have been made, such as the adenoma–carcinoma sequence (8), mutations of K-ras, loss of heterozygosity of 17p, and overexpression of the p53 tumor suppressor protein (9). Defective mismatch repair (MMR), as assessed by microsatellite instability (MSI) and immunohistochemistry, has been consistently found in ~15% of sporadic colorectal carcinomas and 10 to 20% of small bowel adenocarcinomas (9–12). Microsatellites are tandem repeats of simple sequences that occur abundantly and randomly in the human genome. Defective DNA MMR is believed to be responsible for the MSI. Several genes, hMLH1, hMSH2, hMSH3, hMSH6, and hPMS2, are involved in human MMR, with germ-line alterations in hMLH1 and hMSH2 accounting for the majority of the MMR mutations in hereditary nonpolyposis colorectal carcinoma (12, 13). The epigenetic silencing of hMLH1 via hypermethylation has been identified as the most frequent abnormality in sporadic colon (14, 15), gastric (16), and several other human cancers (17). Although the cause of the abnormal methylation in these sporadic cancers is unknown, deficiencies of dietary folate (18) and/or selenium (19) may contribute to these abnormalities. Patients with celiac disease have been shown to have decreased serum levels of folate (20) and selenium (21). These recent findings in small bowel adenocarcinomas and colorectal carcinoma, together with dietary and environmental factors, may provide biological insight into the etiology of the increased risk of small bowel adenocarcinoma in celiac disease.

MSI has been associated with better survival and prolonged disease-free survival in sporadic colon cancer (22–24) and better survival in gastric (25) and pancreas adenocarcinomas (26). Clinically, survival appears to be better for small bowel adenocarcinoma associated with celiac disease compared with sporadic small bowel adenocarcinoma. However because of the rarity of these diseases, there have been only limited case reports detailing the clinical presentations or outcomes (6, 7, 27).

The goals of this study include (a) investigation of the biological causes of small bowel adenocarcinoma in celiac disease, (b) comparison of survival between celiac disease patients and patients without celiac disease with small bowel adenocarcinoma, and (c) characterization of clinicopathologic features of small bowel adenocarcinoma in celiac disease.

MATERIALS AND METHODS

Patients. Of 2085 small bowel adenocarcinomas treated at the Mayo Clinic Rochester from January 1972 to November 2002, 16 patients (incidence 0.8%) with small bowel adenocarcinoma and celiac disease were identified by searching a prospective tumor registry database. One additional celiac disease patient with small bowel adenocarcinoma was identified in the period before 1972 by International Classification of Diseases 9 records. Initially, three age- and gender-matched control patients with sporadic small bowel adenocarcinoma but without celiac disease (control group) were matched to each celiac disease patient (celiac disease group). An additional two controls were obtained for each celiac disease patient that matched by stage, age, and gender. All control patients that matched by stage, age, and gender from the initial control group were added to the group of 34 new controls and these 40 patients made up the stage-matched control group. One celiac disease patient was excluded from the analysis because small bowel adenocarcinoma was diagnosed at autopsy. The initial diagnosis of celiac disease was made by intestinal biopsy in 10 patients (58.8%) and by the surgical specimen in 6 (35.3%). The remaining patient was originally diagnosed with celiac disease by classical symptoms of
malabsorption and characteristic findings on small bowel follow-through studies. This diagnosis was later confirmed by classic features of celiac disease on histologic examination of the nonmalignant mucosa from the small bowel resection. Review of all control specimens demonstrated no evidence of celiac disease. Two patients in the control group had familial adenomatous polyposis and four patients had Crohn’s disease. Clinical staging was performed in accordance with the Union International Contre Cancer and American Joint Committee on Cancer Tumor-Node-Metastasis classifications (28). This retrospective matched case control study was approved by the Institutional Review Board at the Mayo Clinic.

**DNA Preparation and Microsatellite Instability Analysis.** DNA was extracted from all available normal and malignant tissue specimens for genetic analysis from paraffin-embedded tissue. Tissue was cut into 10-μm thick sections and then mounted onto glass slides. One reference slide was stained with H&E. Areas of normal mucosa and tumor were identified and marked; areas of tumor contained >70% cancer cells. The tissue was then scraped into microcentrifuge tubes, and the DNA was extracted using the Qamp Tissue kit (Qiagen, Inc., Santa Clara, CA) according to the manufacturer’s instructions.

Paired normal and tumor DNA were analyzed for MSI with 10 microsatellite markers (6 dinucleotide: DSS346, MYCL, D18S55, D17S250, D10S197, and ACTC; and 4 mononucleotide: BAT25, BAT26, BAT40, and BAT34c) using an ABI 3100 automated nucleic acid analyzer. Tumors were classified as having high-frequency MSI (MSH-H) if ≥30% markers demonstrated instability, low-frequency MSI if <30% demonstrated MSI, and microsatellite stable if no marker exhibited MSI (13). Although all 10 markers were used on each of the normal and tumor pairs, there were some PCR failures due to the degraded nature of the paraffin-embedded tissue. On average, however, at least 8 markers were successfully amplified, and tumors were scored for instability only if 5 of the 10 markers successfully amplified for both the normal and tumor DNA.

**Immunohistochemical Analysis.** The expression of hMLH1 and hMSH2 protein was assessed by immunohistochemistry analysis as described previously (11, 13). Briefly, 5-μm tissue sections from formalin-fixed, paraffin-embedded tissue were stained with antibody to hMLH1 (1 mg/mL, clone G168 728; PharMingen, San Diego, CA) and hMSH2 (0.5 mg/mL, clone FE11, Oncogene Science, Cambridge, MA). Tumor cells that showed an absence of nuclear staining in the presence of normal positive staining in surrounding cells were interpreted as having an absence of expression of these proteins.

**Statistical Analysis.** The association of patient characteristics with case-control status was assessed using logistic regression. A stratified proportional hazards regression analysis, with each strata formed by a set of one celiac disease patient and their matched controls, was used to assess overall survival from the date of the oncologic operation to the date of last follow-up. The association between overall survival and patient status (celiac disease case versus control) was assessed using the estimated regression coefficients from the proportional hazards regression model. Separate models, including stage, age, and interaction terms with patient case-control status were also examined. Kaplan-Meier plots of overall survival for all cases and controls, and separately in early (stage I and II) versus late (stage III and IV) stage cancer, were constructed.

**RESULTS**

**Frequency of Defective Mismatch Repair.** Eleven tumor specimens from the celiac disease group were available for genetic testing. No MSI or abnormal immunohistochemistry staining was identified in the normal mucosa from the small bowel resection. Of the 11 tumors, 2 were microsatellite stable (18%), 1 was classified as low-frequency MSI (9%) with normal protein expression, and 8 tumors (73%) were classified as MSH-H with loss of expression of hMLH1 in 6 and loss of expression of hMSH2 in 1 (Fig. 1). The eighth tumor with MSH-H had intact staining for both hMLH1 and hMSH2. Three patients with MSI-H tumors and absence of hMLH1 expression within the small bowel adenocarcinoma tested negative for germ-line mutations by peripheral blood sampling.

Twenty-two tumor specimens were available for genetic testing from the control groups, 10 from the age- and gender-matched controls and 12 from the stage-matched controls (Fig. 1). No MSI or abnormal immunohistochemistry staining was identified in the normal mucosa from the small bowel resection. Twenty of the tumors (91%) were microsatellite stable and 2 (9%) were MSH-H with loss of hMLH1 expression in both. One MSH-H tumor occurred in each control group.

**Survival.** Stage is a strong prognostic indicator for survival in sporadic small bowel adenocarcinoma; however, there was no association detected between survival and stage of small bowel adenocarcinoma in the celiac disease group alone. Overall, patients with celiac disease had better survival than control patients (median survival 8.1 versus 2.5 years and 5-year survival 64.2% versus 27.4%, P = 0.039, hazard ratio = 27.8, 95% confidence interval, 1.18–657.48; Fig. 2A). However, a case by stage interaction effect was detected (P = 0.017; Fig. 2, B and C). Median survival in early-stage (stage I and II) small bowel adenocarcinoma was 16.1 years (5-year survival, 76.2%) in the celiac disease groups and 10.3 years (5-year survival, 80.0%) in the control group (Fig. 2B). Median survival in late stage (stage III and IV) small bowel adenocarcinoma was 6.1 years (5-year survival, 51.4%) in the celiac disease group and 1.4 years (P = 0.025; Fig. 3). Tumor location within the small bowel was not associated with survival by Cox proportional hazards analysis (P = 0.331, hazard ratio = 1.76, 95% confidence interval, 0.56–5.57).

**Characteristics of Small Bowel Adenocarcinoma.** Relevant demographic and clinicopathologic data for celiac disease and control patients are summarized in Table 1. The diagnosis of celiac disease was made postoperatively by retrospective review of either the operative specimen or a postoperative biopsy in seven patients (41.2%). Two of these patients were diagnosed by endoscopic small bowel biopsy and 3 were diagnosed by retrospective review of the small bowel resection specimen. All operative specimens had classic features of celiac disease on retrospective review. The most common presentation for celiac disease was diarrhea (16 patients, 88.9%) and weight loss (9 patients, 50%) and for small bowel adenocarcinoma in the celiac disease group was intestinal obstruction (11 patients, 64.7%) and weight loss with abdominal pain (6 patients, 35.3%). Adenocarcinoma stage was associated with the presence of celiac...
Disease (P = 0.018), with early-stage (stage I and II) small bowel adenocarcinoma and early T stage (T1 or T2) more common in the celiac disease group when compared with the control patients (Table 1). Demographic information for celiac disease patients with MSI-H tumors is summarized in Table 2. In general, the majority of celiac disease patients with MSI-H tumors were diagnosed with celiac disease preoperatively; however, none of these patients followed a gluten-free diet strictly. Tumors that were MSI-H were larger were frequently associated with lymph node metastases and demonstrated tumor extension into mesenteric vessels or adjacent organs.

**DISCUSSION**

There are two novel observations in this study: first, the unusually high frequency of defective MMR in celiac disease patients with adenocarcinoma of the small bowel; and second, the better survival for adenocarcinoma of the small bowel in celiac disease patients compared with sporadic small bowel adenocarcinoma. The very high frequency of defective MMR in these patients raised the concern that these tumors were familial. However, in tumors with loss of hMLH1 protein expression, the defect appears to be the result of somatic rather than germ-line mutations. We were able to demonstrate this in three patients by testing peripheral blood specimens.
Previously, the frequency of defective MMR in most other sporadic tumors examined is on the order of 10 to 20%. The finding of defective MMR in 73% of small bowel adenocarcinoma in celiac disease patients is, therefore, very unusual. The biological explanation for this unusually high frequency is not known but may provide important insight into the mechanisms of tumor initiation involving the MMR pathways for these and other cancers. MSI has been demonstrated in several other lesions associated with inflammation, including chronic ulcerative colitis-associated dysplasia (19 to 46%) and ulcerative colitis-associated colon cancer (21 to 40%; refs. 29–31), lymphomas of mucosal-associated lymphoid tissue (53%; ref. 32), Barrett’s associated adenocarcinoma of the esophagus (22%; ref. 33), and nonfamilial colorectal cancers with intraepithelial lymphocytes (9%; ref. 34). The observation of MSI in regenerating intestinal mucosa may reflect additive effects of ongoing DNA damage that occurs in the epithelium as a result of chronic inflammation (33). Chronic inflammation may lead to abnormal methylation, which in the case of hMLH1, leads to epigenetic silencing. Inactivation of the MMR genes may then lead to an increase in additional DNA replication errors and/or the loss of the apoptotic pathway.

We hypothesize that the increased frequency of defective MMR in celiac disease patients who have small bowel adenocarcinoma may be caused by the inflammation of the intestine resulting from intolerance to dietary gluten. This inflammation results in rapid turnover of the mucosal layer of the small intestine, which may allow for high rates of spontaneous mutations or epigenetic silencing of the MMR genes, resulting in defective MMR. A second possibility is that malabsorption by the inflamed mucosa results in vitamin deficiencies, which may in turn, lead to inactivation of the MMR pathway. Low folate status has been suggested as an additional cause of MSI in patients with ulcerative colitis, and folate supplementation has been associated with improvements in the MSI pattern (35). A third possible mechanism is that the small bowel inflammation does not result in dysplasia but follows the traditional adenoma-carcinoma sequence. Indeed, a previous study from our institution identified a thin rim of dysplasia at the tumor margin in 50% celiac disease patients; however, there was no flat dysplasia of the mucosa extending from the tumor margin or distant from the carcinoma (5). Six of our current patients were included in this study’s cohort. Recent evidence supports the adenoma-carcinoma sequence (36); however, no adenomas were found in our patients. The exact mechanism leading to loss of MMR function is at this point not known. Regardless of the true mechanism, defective MMR appears to result in excellent patient survival with a more favorable tumor biology in patients with celiac disease. These findings and the unique features of celiac disease may provide insight on the effects of diet, inflammation, and the biology of other gastrointestinal cancers.

Initially, we matched the control patients by age and gender only. Statistical analysis revealed an association with survival by case status, but stage was a strong indicator of survival as well. Two additional age-, gender-, and stage-matched control patients per celiac disease patient were obtained, and a reanalysis examined all stage-matched control patients, including those that matched previously by stage from the first set of controls. This analysis indicated a survival advantage for patients with small bowel adenocarcinoma and celiac disease compared with patients with small bowel adenocarcinoma alone. Despite our diligence in identifying and matching control patients, the small number of patients in the celiac disease group creates a lack of power in the statistical analysis. Multiple control patients were matched per celiac disease patient to offset this lack of power. However, the Kaplan-Meier survival plots for the control patients may reflect the selection process and not that of small bowel adenocarcinoma patients in general. Also, control patients were not matched for the location of tumor within the small bowel. Regression analysis demonstrated that tumor location was not a significant predictor of survival in the celiac disease and control patients. Despite these limitations, the 5- and 10-year survivals for small bowel adenocarcinoma in the celiac disease patients are excellent, and no association between survival and stage of small bowel adenocarcinoma was detected in the 17 celiac disease patients, although there was limited power with just seven events. Stage is generally a strong prognostic indicator for survival in sporadic small bowel adenocarcinoma (22, 37). Regardless of the control patients, MMR abnormalities and MSI may be important prognostic indicators in celiac disease patients with intestinal adenocarcinomas and for other gastrointestinal cancers. Such genetic testing may allow for individualized treatment plans and chemotherapy protocols (38, 39).

We can speculate that adherence to a strict gluten-free diet may reduce the increased risk of small bowel adenocarcinoma in patients who have celiac disease. However, entirely eliminating gluten from one’s diet is nearly impossible. Routine screening of patients with celiac disease for small bowel adenocarcinoma or other malignancies associated with celiac disease is not currently recommended. Patients who have celiac disease present with lower stage small bowel adenocarcinoma than control patients, and the most common presenting symptom of small bowel adenocarcinoma is obstruction. Thus, any patient with celiac disease that presents with symptoms of obstruction, weight loss, or abdominal pain should undergo evaluation of their intestinal tract for the possibility of malignancy. If a malignancy is discovered, aggressive surgical and medical therapy is justified because of the excellent 5- and 10-year survival. Additionally, all patients that undergo operation for small bowel adenocarcinoma should be evaluated for celiac disease by inspection of the surgical specimen, serologic testing, and/or an intraoperative biopsy of the duodenum because many of the patients in this study were diagnosed with celiac disease after their oncologic procedure. Although it is not clear whether the identification of celiac disease and treatment with a gluten-free diet will prevent cancer reoccurrence, treatment with a gluten-free diet can decrease the incidence of intestinal morbidity postoperatively.

In conclusion, patients who have small bowel adenocarcinoma and celiac disease have a high frequency of defective MMR and appear to have improved survival compared with patient who have sporadic small bowel adenocarcinoma. Although the link between environmental factors such as diet and cancer biology may be accentuated in the celiac disease population, these findings may have relevance in other types of gastrointestinal cancers such as sporadic colorectal and gastric cancers.

Table 2  Characteristics of MSI-H unstable tumors in patients with celiac disease

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MSI-H (n = 8)</th>
<th>MSS and MSI-L (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of small bowel adenocarcinoma, years (range)</td>
<td>61.7 (42–78)</td>
<td>59.9 (42–71)</td>
</tr>
<tr>
<td>Age of celiac disease diagnosis, year (range)</td>
<td>53.9 (25–77)</td>
<td>53.3 (36–72)</td>
</tr>
<tr>
<td>Preoperative celiac disease diagnosis (%)</td>
<td>62.5 (5)</td>
<td>33.3 (1)</td>
</tr>
<tr>
<td>Strict gluten-free diet (%)</td>
<td>0 (0)</td>
<td>33.3 (1)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>37.5 (3)</td>
<td>66.7 (2)</td>
</tr>
<tr>
<td>Duodenal primary tumor (%)</td>
<td>25 (2)</td>
<td>100 (3)</td>
</tr>
<tr>
<td>Jejunal primary tumor (%)</td>
<td>75 (6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Stage of cancer (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>12.5 (1)</td>
<td>16.7 (2)</td>
</tr>
<tr>
<td>2</td>
<td>37.5 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>3</td>
<td>25 (2)</td>
<td>33.3 (1)</td>
</tr>
<tr>
<td>4</td>
<td>25 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Tumor grade</td>
<td>2.5 (n = 6)</td>
<td>3</td>
</tr>
<tr>
<td>Tumor size &gt; 4 cm (%)</td>
<td>75 (6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Tumor extension (%)</td>
<td>62.5 (5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Lymph node metastases (%)</td>
<td>50.0 (4)</td>
<td>33.3 (1)</td>
</tr>
</tbody>
</table>

Abbreviations: MSS, microsatellite stable; MSI-L, low-frequency microsatellite instability.
ACKNOWLEDGMENTS

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REFERENCES

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