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 (MSI-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 status) 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 DefectiveMismatch 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 MSI-H with loss of expression of hMLH1 in 6 and loss of expression of hMSH2 in 1 (Fig. 1). The eighth tumor with MSI-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 MSI-H with loss of hMLH1 expression in both. One MSI-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 14 years (5-year survival, 13.4%) in the controls (Fig. 2C). Therefore, a subset analysis including just stage-matched controls was examined to eliminate the stage effect for the improved survival in the celiac disease group. After stratifying by matched sets, the Cox proportional hazards analysis detected a significant association of case status with survival compared with the stage-matched controls (median survival 8.1 versus 1.4 years and 5-year survival 64.2 versus 26.1%, 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 \((T_1 \text{ or } T_2)\) 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.

**Fig. 2.** A, Kaplan-Meier survival estimates for the overall survival of patients with small bowel adenocarcinoma. B, Kaplan-Meier survival estimates for survival of patients with stages I and II small bowel adenocarcinoma. C, Kaplan-Meier survival estimates for survival of patients with stages III and IV small bowel adenocarcinoma. Overall, patients with celiac disease (CD) had better survival compared with patients with sporadic small bowel adenocarcinoma. However, a case-by-stage interaction effect was detected \((P = 0.017)\).

**Fig. 3.** Kaplan-Meier survival estimates for survival of celiac disease (CD) patients and stage-matched controls with small bowel adenocarcinoma.

<table>
<thead>
<tr>
<th></th>
<th>Celiac disease ((n = 17))</th>
<th>Controls ((n = 51))</th>
<th>Stage-matched controls ((n = 40))</th>
</tr>
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<tbody>
<tr>
<td>Age of small bowel adenocarcinoma, years (range)</td>
<td>59.5 (42–78)</td>
<td>58.8 (30–87)</td>
<td>62.9 (38–84)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>47.1 (8)</td>
<td>47.1 (24)</td>
<td>37.5 (15)</td>
</tr>
<tr>
<td>Stage of cancer (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>23.5 (4)</td>
<td>2.0 (1)*</td>
<td>15.0 (6)</td>
</tr>
<tr>
<td>2</td>
<td>29.5 (5)</td>
<td>19.6 (10)*</td>
<td>27.5 (11)</td>
</tr>
<tr>
<td>3</td>
<td>23.5 (4)</td>
<td>51.0 (26)*</td>
<td>35.0 (14)</td>
</tr>
<tr>
<td>4</td>
<td>23.5 (4)</td>
<td>27.4 (14)*</td>
<td>22.5 (9)</td>
</tr>
<tr>
<td>Site of cancer (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>47.1 (8)</td>
<td>54.9 (28)</td>
<td>75.0 (30)</td>
</tr>
<tr>
<td>Jejunum</td>
<td>52.9 (9)</td>
<td>33.3 (17)</td>
<td>15.0 (6)</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.0 (0)</td>
<td>11.8 (6)</td>
<td>10.0 (4)</td>
</tr>
<tr>
<td>Pathology (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade</td>
<td>3.0</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>(T1)</td>
<td>5.9 (1)</td>
<td>0.0 (0)*</td>
<td>12.8 (5)</td>
</tr>
<tr>
<td>(T2)</td>
<td>17.6 (3)</td>
<td>0.0 (0)*</td>
<td>12.8 (5)</td>
</tr>
<tr>
<td>(T3)</td>
<td>41.2 (7)</td>
<td>47.9 (23)*</td>
<td>23.1 (9)</td>
</tr>
<tr>
<td>(T4)</td>
<td>35.3 (6)</td>
<td>52.1 (25)*</td>
<td>51.3 (20)</td>
</tr>
<tr>
<td>Size &lt; 2 cm</td>
<td>5.9 (1)</td>
<td>2.0 (4)</td>
<td>27.5 (11)</td>
</tr>
<tr>
<td>Size &gt; 4 cm</td>
<td>52.9 (9)</td>
<td>49.0 (25)</td>
<td>32.5 (13)</td>
</tr>
<tr>
<td>Lymph node metastases</td>
<td>47.1 (8)</td>
<td>60.8 (31)</td>
<td>47.5 (19)</td>
</tr>
<tr>
<td>Cancer-related deaths (%)</td>
<td>17.6 (3)</td>
<td>66.7 (34)</td>
<td>70.0 (28)</td>
</tr>
<tr>
<td>Operation for cure (%)</td>
<td>82.4 (14)</td>
<td>60.8 (34)</td>
<td>70.0 (28)</td>
</tr>
</tbody>
</table>

* \(P < 0.05\) by logistic regression compared with celiac disease group.
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 important insight into the mechanisms of tumor initiation involving 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, celiac disease patients that undergo operation for 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 gastrointestinal cancers.

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.
ACKNOWLEDGMENTS

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REFERENCES

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

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