High Epithelial and Stromal Genetic Instability of Chromosome 17 in Ulcerative Colitis-associated Carcinogenesis

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Abstract
To define the relative frequencies of genetic instability in stromal and epithelial compartments during ulcerative colitis (UC)-associated tumorigenesis, samples from laser-captured microdissection were assessed for microsatellite instability and loss of heterozygosity in regenerative tissue, dysplasia, and carcinomas in long-standing UC cases. Five National Cancer Institute-recommended standard markers and four markers located close to p53 and BRCA1 genes in chromosome 17 were tested, and p53 gene sequencing was also carried out. Although chromosome 17-MSI and -loss of heterozygosity in epithelium correlated with histological progression, in stroma they showed a consistently high frequency throughout the different stages, indicating a distinct carcinogenesis pathway of UC. The rates for standard markers were lower in both epithelium and stroma.

Introduction
Interactions between epithelial and mesenchymal cells in organs play important roles in their development, differentiation, and growth (1). However, the contribution of stromal cells to generation and progression of epithelial neoplasia has not been thoroughly investigated (2, 3). Previously, we revealed that not only epithelium but also stroma may demonstrate genetic instability and that such stromal alteration might influence epithelial tumorigenesis in S-CRC-3 cases (4), using a combination of novel microdissection and MSI testing with markers close to the p53 and BRCA1 genes on Chr.17. Thus, we have evidence that our selected Chr.17 markers may be clinically useful. In the S-CRC, p53 gene alteration generally occurs in late stages, whereas in UC-carcinogenesis, in contrast, it appears to be an early event (5, 6), suggesting differences in the lineage. Why this might be the case, and the involvement of stromal and epithelial elements, was primary focus of the present study. Another question remains of how MSI detection with Chr.17 markers compares with that using NCI-recommended Std MSI markers. Therefore, both NCI-Std markers (7) and Chr.17 markers in epithelium and stroma were here analyzed in a series of microdissected samples of regenerative mucosa, IND, low-grade dysplasia, HGD, and cancers in UC cases, the results then being compared with our previous data for S-CRC cases (4).

Materials and Methods

Samples. Nine colorectal specimens were obtained from patients with UC-associated tumors, containing of 50 lesions [10 regenerative mucosae, 7 IND, 18 low-grade dysplasias, 4 HGDs, and 11 invasive carcinomas (UC-Ca); Ref. 8]. The UC cases were long standing (total duration, 6–20 years; age, 21–64 years old). In addition, 10 samples of noninflammatory normal mucosae from S-CRC cases were obtained for comparison. Serial 10-μm-thick sections were used for microdissection, and serial 3-μm-thick sections were applied for H&E staining and immunohistochemistry after deparaffinization. This allowed p53 gene and protein alterations to be analyzed for individual lesions (Fig. 1A).

DNA Extraction. Microdissection and DNA extraction were carried out as described previously (4). Briefly, epithelial and adjacent stromal tissues in the lamina propria were carefully microdissected with a laser-captured microdissection system (LM200; Arcturus, Mountain View, CA) to avoid cross-contamination (Fig. 1B). Normal control DNA for each lesion was obtained from histologically normal lymph nodes.

Microsatellite Analysis. The PCR was performed for four microsatellite markers selected for analyzing allelic instability in Chr.17 (dinitucleotide repeat), D17S796, TP53, D17S786 (close to the p53 gene), and D17S579 (close to BRCA1) and five NCI-Std markers for S-CRC analysis, BAT25, BAT26 (mononucleotide repeat), D2S123, D5S346, and D17S250 (dinitucleotide repeat; Ref. 7). MSI and LOH were detected by the PCR-single strand conformation polymorphism method as described previously (Ref. 4; Fig. 1C). Representative data were confirmed by ABI PRISM 310 Genetic Analyzer, GeneScan Analysis software (version 3.7.1), and Genotyper software (version 3.7; PE Applied Biosystems, Foster City, CA). “MSI+/LOH+ lesion” refers to a lesion showing MSI+/LOH+ for at least one marker.

p53 Gene Analysis and Immunohistochemistry. p53 mutations and p53 protein expression were analyzed as described previously by ourselves (5). Briefly, p53 mutations were detected in single crypts by PCR-direct sequencing. Immunohistochemical staining was performed with monoclonal anti-p53 (DO7, ×300 dilution; Novocastra Lab, Newcastle, United Kingdom), anti-hMLH1 (Clone; G168-15, ×200 dilution; BD PharrMingen, San Diego, CA), and anti-hMSH2 (Clone; G219-1129, ×500 dilution; BD PharrMingen) antibodies, using the Std-labeled streptavidin-peroxidase complex method with microwave treatment as detailed earlier (5). The amounts of positive cells were expressed as the percentage of the total number of epithelial cells and assigned to one of three categories for p53; +, diffusely positive; +, +, positively staining of the lower half of the crypt; ±, scattered staining; −, negative; and for hMLH1 and hMSH2: +, +, +, >50%; +, 10–50%; −, <10%.

Statistics. Differences were examined using Fisher’s exact and χ2 tests, as well as the nonparametric Mann-Whitney U test.

Results

MSI and LOH Status in UC-associated Lesions. Chr.17 MSI positivity (Chr.17 MSI+) for one or more of the markers was detected frequently in both epithelium (37%) and adjacent stromal areas (43%; Table 1). Of the total of 50 lesions, 57% were Chr.17 LOH positive (Chr.17 LOH+) for one or more of the markers in epithelium and 8.2% in stroma. Epithelial Chr.17 LOH + for TP53 (46%) was relatively more often found than for the other markers (Table 2).

Compared with the Chr.17 results, MSI and LOH on Std markers (Std MSI+, Std LOH+) were significantly less frequent in UC-associated lesions (MSI+ in epithelium, P = 0.0055; in stroma,
MSI and LOH Status, p53 Gene Mutations, and p53 Protein Overexpression in UC-associated Lesions. A significant correlation between epithelial p53 protein overexpression and Chr.17 LOH+ was found in UC-associated lesions ($P = 0.0033$; Table 1). p53 gene mutations were detected in 20 of 48 (42%) lesions. No relation was found between epithelial p53 mutation and epithelial or stromal MSI and LOH.

hMLH1 and hMSH2 Protein Expression in Epithelium in UC-associated Lesions. Losses of epithelial hMLH1 and hMSH2 protein expression were infrequent and not significantly related to epithelial or stromal MSI or LOH (Table 1).

LOH Frequencies on Chr.17 Markers in UC-Ca Versus S-CRC. Epithelial Chr.17 LOH+ was more frequent in UC-Ca than in the S-CRC, described in our previous report (4). Epithelial LOH for D17S579 was also significantly more frequent in UC-Ca than in S-CRC (Table 2).

Discussion

In the present study, with Chr.17 MSI markers, frequencies of stromal MSI were consistently high through progression from regenerative mucosa, whereas the levels of MSI and LOH of Chr.17 in epithelium of UC-associated lesions correlated loosely with histological progression. Interestingly, compared with epithelial Chr.17 LOH+ lesions without p53 protein overexpression (−), those with p53 overexpression (±, +, and + +) had a significant relation with histological progression of UC-associated lesions. The results strongly suggest that stromal genetic instability precedes and influences epithelial tumorigenesis, with stromal tissue under the inflammation-stressed conditions featuring Chr.17 MSI from an early stage. On the contrary, it is interesting that malignant phyllodes tumors had distinct genetic changes in both stroma and epithelium (9), and epithelial genetic changes preceded those of stroma in breast carcinomas (10, 11). In UC, epithelial genetic changes, including MSI, are reported in nonneoplastic mucosa (12, 13), but stromal genetic changes had not been reported. In line with our results, it is reported that inflammation-associated stroma promotes conversion of colonic adenoma cells to adenocarcinoma cells in nude mice (14).

Although MSI/LOH in UC-associated lesions was here found to be less frequent with Std than with Chr.17 markers, epithelial Std MSI was often detected in UC-Ca lesions, indicating occurrence as a late event in UC carcinogenesis. Our results indicate that Chr.17 markers may be more useful to detect genetic instability of UC-associated lesions involved in dysplasia-carcinoma development, compared with Std markers. Possible reasons for high sensitivity of our selected Chr.17 markers can be proposed: (a) loci adjacent to the p53 gene tend to be exposed to risk of mutation/genetic instability; (b) because all of our selected markers are dinucleotide-repeats ([CA]n), whereas NCI-Std markers include two mononucleotide-repeats, the latter might be less sensitive for detection of MSI or LOH.

It has been reported that Std MSI-high (-H) cancers feature loss of hMLH1/hMSH2 protein in S-CRC (15) and are sensitive to be detected by mononucleotide repeat markers (16). In recent studies using Std markers (17, 18), whereas S-CRC with Std MSH-H showed a ~80% hMLH1 hypermethylation rate, only 46% of UC-Ca with Std MSI-H demonstrated hMLH1 hypermethylation. Furthermore, MSI-H with loss of hMLH1/HMSH2 is infrequent in UC-Ca (18). In agreement, our study showed no significant relation between Chr.17/Std MSI and loss of hMLH1/HMSH2 in UC-associated tumor lesions.

Comparison of LOH frequencies between Chr.17 markers revealed epithelial Chr.17 LOH+ for TP53 to be most frequently encountered (Table 2). Furthermore, epithelial LOH for D17S579 was significantly more frequent in UC-Ca than in S-CRC lesions, which might be
caused by the difference of MSI generating mechanism in UC and S-CRC cases, including DNA repair enzymes. Interestingly, TP53 and D17S579 (localized adjacent to BRCA1 gene) LOH, detected more frequently in UC-Ca than in S-CRC, is often identified in breast and ovarian cancers (19). The product of the BRCA1 putative tumor suppressor gene interacts directly with p53 and transcriptionally activates p21 (20). Therefore, together with the p53 gene, alteration in the BRCA1 gene could contribute to DNA repair system underlying genetic instability in UC.

From the available data, we propose that Chr.17 markers, located near the p53 and BRCA1 genes, are useful for identifying genetic instability in UC lesions in the pathway to neoplastic development. We also suggest that stromal abnormality in Chr.17 MSI influences epithelial tumorigenesis involving epithelial Chr.17 MSI/LOH and p53 protein overexpression.

Table 1  Chromosome17 MSI/LOH and Std MSI/LOH findings for epithelium and stroma in UC-associated lesions

<table>
<thead>
<tr>
<th>Chr. markers (D17S796, TP53, D17S786, and D17S579)</th>
<th>NCi-Std Markers (BAT25, BAT26, D2S123, D5S346 and D17S250)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chr.17 markers: REG, IND, LGD, HGD, Ca.</td>
<td>MSI+/informative lesions (%)</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>Total P</td>
<td>Stroma</td>
</tr>
<tr>
<td>50</td>
<td>18/49 (37)</td>
</tr>
<tr>
<td>7</td>
<td>1/7 (14)</td>
</tr>
<tr>
<td>18</td>
<td>12/18 (67)</td>
</tr>
<tr>
<td>4</td>
<td>4/4 (100)</td>
</tr>
<tr>
<td>11</td>
<td>2/10 (20)</td>
</tr>
<tr>
<td>6/10 (60)</td>
<td>8/10 (80)</td>
</tr>
</tbody>
</table>

p53 mutation

+ 20 | 6/20 (30) | 2/20 (10) | 0/20 (0) | 1/18 (5.6) | 0/18 (0) | 1/18 (5.6) |
| 28 | 12/28 (43) | 8/28 (29) | n.s. | 4/28 (14) | n.s. | 4/28 (14) |
| 18 | 7/18 (39) | 9/18 (50) | n.s. | 5/18 (28) | 0/18 (0) | 5/18 (28) |
| 2 | 1/2 (50) | 1/2 (50) | n.s. | 2/2 (100) | 0/2 (0) |
| 12 | 5/12 (42) | 6/12 (50) | n.s. | 11/12 (92) | 0.0006 | 3/12 (25) |
| 17 | 5/17 (29) | n.s. | 5/17 (29) | 0/17 (0) | n.s. | 5/17 (29) |

hMLH1 protein

1/1 (100) | 1/1 (100) | 0/1 (0) | 2/9 (22) | 3/9 (33) | 0.0323 |
| 5 | 2/5 (40) | 5/5 (100) | n.s. | 0/5 (0) |
| 39 | 12/33 (36) | n.s. | 15/33 (46) | n.s. | 3/28 (11) | n.s. | 4/18 (24) |

hMSH2 protein

1/1 (100) | 1/1 (100) | 0/1 (0) | 0/1 (0) |
| 2 | 0/2 (0) | 1/2 (50) | 0/2 (0) |
| 43 | 14/43 (33) | n.s. | 16/43 (37) | n.s. | 3/37 (8.1) | n.s. | 5/37 (14) |

References


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