CELL PROLIFERATION IN ESOPHAGEAL COLUMNAR EPITHELIUM
(BARRETT’S ESOPHAGUS)

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In order to correlate histopathology and cell turnover in esophageal tissue in patients
with esophageal columnar epithelium, biopsy specimens from 11 patients were studied.
Two of the patients had adenocarcinoma of the esophagus not present in the specimens
studied. In the heterogeneous esophageal columnar epithelium, the DNA synthesis
phase averaged 10.4 ± 0.3 hr; labeling index of cells in the crypt-like structures was
23.3 ± 0.3%; generation time was 44.6 ± 1.2 hr, and the mitotic index was 1.82 ± 0.07%.
Significant differences were not present among specimens with columnar epithelium
but they differed from cell proliferation in adjacent squamous epithelium where S phase
was similar but labeling and mitotic indexes were lower and generation time increased
to 105 ± 6 hr. In the 2 patients with adenocarcinoma of the esophagus, and in 1 of 9
without adenocarcinoma, labeling of surface columnar cells after brief exposure to
[3H]thymidine was present and suggests that this may be a manifestation of early
malignant change.

Columnar epithelium in the distal esophagus is associated with esophagitis, midesophageal stricture, deep
ulceration, and occasionally adenocarcinoma. The epithelium is a heterogeneous tissue which may contain
cells similar to gastric and intestinal tissues. Animal studies and observations in man suggest that the tissue
develops as a response to reflux esophagitis with subsequent regeneration of immature gastric cells that may
undergo metaplastic changes. Thirty-two cases of invasive carcinoma and 3 cases of carcinoma in situ
have been reported with this condition, and surveillance for early signs of malignant transformation has been
recommended. Histological criteria for epithelial dysplasia and carcinoma in situ have been described
but the relationship between these changes and future behavior is uncertain. To determine whether differences
in cell renewal exists between squamous epithelium and columnar epithelium demonstrating a spectrum of
histopathology, the kinetics and distribution of cell proliferation were determined in esophageal speci-
mens from 11 patients with columnar esophageal epithelium.

Methods

Patients. Eight men and 3 women ranging in age from 36 to 71 years were diagnosed as having esophageal columnar
epithelium (Barrett’s esophagus) on the basis of histopathological examination of multiple esophageal biopsies obtained via
fiberoptic endoscopy at the University of Utah Medical Center. After obtaining written informed consent, biopsies of the
esophageal columnar and squamous epithelia were obtained for cell proliferation studies at the time of follow-up esopha-
goscopy. All of the patients had symptoms of chronic acid-peptic reflux esophagitis exceeding 10 years, manometrically
demonstrated low distal esophageal sphincter pressure, esophageal reflux of 0.1 N HCl instilled into the stomach, and
endoscopic and histological evidence of esophagitis. Deep esophageal ulcers were present in 5 patients, and 6 patients
had midesophageal strictures. One patient (J. R.) had evidence of multiple foci of severe dysplasia and adenocarcinoma
in situ. He underwent esophagectomy with colonic interposition but a 2- to 2-cm patch of columnar epithelium, at the
level of the carina, was not included in the resection. Biopsies were obtained from this site for cell proliferation studies. One
patient (R. S.) had evidence of adenocarcinoma involving the esophageal columnar epithelium at the time of endoscopy and
biopsy. Subsequent resection of the esophagus showed several areas of adenocarcinoma in situ with one focus of microinva-

Tissue studies. Biopsies were prepared for routine histology by staining with hematoxylin and eosin. Mitotic indexes
(MI’s) were determined by counting at least 1000 epithelial cells. Classification of the histological type of tissue was made
diagnosis of the basis of criteria utilized for colonic tissue. Determining gradations of epithelial dysplasia is necessarily
subjective and arbitrary; no epithelial dysplasia and severe dysplasia are more easily defined than mild and moderate
gradations. The coded histological sections in this study were reviewed independently by two investigators (M. B., D. M.)
with excellent correlation. Differences, where they occurred, were resolved by averaging the values. Dysplasia was as-
signed a grade according to the following criteria: 0, no epithelial dysplasia; 1, minimal change characterized by slight
pleomorphism and nuclear irregularities; 2, moderate change indicated by more prominent nuclear and nucleolar abnormalities;
and 3, severe epithelial dysplasia indicated by marked irregularities of nuclear and nucleolar size and composition.
pseudostratification of nuclei, abnormal mitoses, and loss of polarity. Carcinoma in situ was diagnosed if a “back-to-back” arrangement of atypical glands was present in addition to severe dysplasia. The degree of inflammation was indicated as follows: 0, no inflammation; 1, mild chronic inflammatory cell infiltrate; 2, moderate chronic and acute cell infiltration; and 3, marked acute and chronic cell infiltration with crypt abscesses.

Cell proliferation studies were performed using a modification of the in vitro double-labeling technique adapted to humans by Galand et al.² This technique has been shown to have a high degree of correlation with more classical in vivo labeling techniques.²³⁻²⁵ Three or four biopsies of esophageal columnar epithelia obtained via fiberoptic endoscopy were immediately placed in cold Eagle’s minimal media supplemented with 10% fetal calf serum. The specimens were cut into 0.5- to 1.0-mm sections. Within 5 min of obtaining the biopsies, the small fragments were placed in a 25-ml Erlenmeyer flask containing 2 ml of Eagle’s minimal media containing 10% fetal calf serum and 1 μCi of [3H]thymidine (6.7 μCi per mmole, New England Nuclear, Boston, Mass.) adjusted to pH 7.2 to 7.4 in an atmosphere of 95% O₂ and 5% CO₂ at 37°C. After a 15-min incubation the tissue fragments were removed and placed in 2 ml of medium free from [3H]thymidine for 30 or 60 min. Finally, they were incubated for 15 min in a flask with 2 ml of medium containing 10 μCi of [3H]thymidine. The tissue fragments were then fixed in 10% buffered formalin and serially sectioned. The slides were dipped in Ifford K-5 gel and exposed for 4 weeks at 5°C. Finally, the slides were developed and counterstained with hematoxylin and eosin.

The slides were coded and the sections examined for the presence of labeled nuclei. The cells were easily identified as being either lightly or heavily labeled with [3H]thymidine. From the formula N₀/Nₐ = S/t, the DNA synthesis phase (S) was calculated. N₀ represented the number of heavily labeled cells, Nₐ the number of weakly labeled cells, and t the interval between the two pulses of [3H]thymidine. Half of the tissue fragments from each of the biopsies were incubated for 30 min and half were incubated for 60 min in the cold medium. In all instances at least 2000 labeled cells were counted per patient. The generation time (GT) was calculated from the formula, GT = S/LI × 100. S is the synthesis phase in hours as calculated above and LI is the labeling index or percentage of cells labeled with [3H]thymidine.

**Results**

The histological type and degree of inflammation and dysplasia noted in the esophageal columnar epithelial biopsies are listed in table 1. Of the 11 patients studied, 9 had intestinal type, 1 had junctional type, and 1 had gastric type epithelium. The two independent observers had complete agreement as to tissue type. This was agreement of opinion as to the degree of inflammation in 7 of 11 cases and they differed by only 1 unit in 4 cases. In these cases the average value is listed. There was concurrence on the degree of dysplasia in 10 of the 11 cases. All of the squamous cell biopsies studied had evidence of esophagitis, including increased thickness of the basal layer and extension of the dermal pegs at least three-fourths of the way to the surface.²⁸

In all cases the columnar esophageal tissue demonstrated crypt-like structures. These structures were surrounded by villous-like structures in intestinal-type epithelium. In junctional or gastric type tissues, they were surrounded by what appeared to be rudimentary villi. Examination of slides showed that mitotic figures were only detected in the crypt-like structures. These structures, in which approximately one-fifth of the cells were labeled with [3H]thymidine, were thought to represent the proliferating compartment, and parameters of cell proliferation were calculated for this group of patients.

**Table 1. Histology and cell kinetics in esophageal columnar epithelium**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Histological type</th>
<th>Degree of inflammation</th>
<th>Degree of dysplasia</th>
<th>Cell kinetics studies</th>
<th>Surface cells labeled</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. G.</td>
<td>Intestinal</td>
<td>1.0</td>
<td>0</td>
<td>h₉</td>
<td>%</td>
</tr>
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<td>W. A.</td>
<td>Intestinal</td>
<td>1.5</td>
<td>0</td>
<td>11.2</td>
<td>23.0</td>
</tr>
<tr>
<td>D. B.</td>
<td>Intestinal</td>
<td>1.0</td>
<td>0</td>
<td>11.1</td>
<td>25.5</td>
</tr>
<tr>
<td>G. W.</td>
<td>Intestinal</td>
<td>1.0</td>
<td>0</td>
<td>10.5</td>
<td>23.1</td>
</tr>
<tr>
<td>S. R.</td>
<td>Intestinal</td>
<td>1.5</td>
<td>1</td>
<td>11.0</td>
<td>21.6</td>
</tr>
<tr>
<td>R. S.</td>
<td>Junctional</td>
<td>1.5</td>
<td>3</td>
<td>10.5</td>
<td>24.1</td>
</tr>
<tr>
<td>V. M.</td>
<td>Intestinal</td>
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<td>0</td>
<td>11.6</td>
<td>23.6</td>
</tr>
<tr>
<td>E. B.</td>
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<td>0</td>
<td>10.7</td>
<td>22.9</td>
</tr>
<tr>
<td>A. H.</td>
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<td>0</td>
<td>9.1</td>
<td>22.2</td>
</tr>
<tr>
<td>D. C.</td>
<td>Gastric</td>
<td>1.0</td>
<td>0</td>
<td>9.1</td>
<td>22.2</td>
</tr>
<tr>
<td>J. R.</td>
<td>Intestinal</td>
<td>1.0</td>
<td>0.5</td>
<td>9.3</td>
<td>24.4</td>
</tr>
</tbody>
</table>

| Mean    |                   |                      |                     | 10.4 | 23.3 | 44.6 | 1.82 |                     |
| SEM     |                   |                      |                     | 0.3  | 0.3  | 1.2  | 0.07 |                     |

* Abbreviations as in table 1.

See text for criteria determining degree of inflammation and dysplasia.

S, synthesis; LI, labeling index; GT, generation time; MI, mitotic index.

Patient with adenocarcinoma.
cells. In biopsies from the 2 patients who had adenocarcinoma detected in other areas of the esophagus (J. R. and R. S.), and 1 other patient (A. H.), occasional cells beyond the crypt-like structures were noted to be labeled. These were infrequent and not contiguous with the rest of the labeled cells and for purposes of calculating cell kinetics were not considered to be part of the proliferative compartment (fig. 1). In the case of squamous epithelium only the 2 basal layers of cells incorporated [³H]thymidine and this was considered the proliferative compartment.

In columnar epithelium the S phase averaged 10.4 ± 0.3 hr, the LI was 23.3 ± 0.3%, GT was 44.6 ± 1.2 hr, and the MI was 1.28 ± 0.07%. Although there were no gross differences in the cell kinetics studies between the 2 patients with adenocarcinoma of the esophagus and the other patients, 1 of the patients with carcinoma had the shortest GT and the other had the highest MI of all patients (table 1). In squamous epithelium the cell turnover was much slower, although the S phase was similar to columnar tissue at 10.6 ± 0.2 hr (table 2). The LI was only 10.0 ± 0.5 hr and the GT was 105 ± 6
hr. This slower generation time was reflected in a low MI, 0.85 ± 0.04%.

Discussion

Columnar-lined esophageal mucosa has been considered a premalignant lesion and careful surveillance for malignant changes has been recommended. Experience with ulcerative colitis suggests that severe dysplasia in colonic biopsies is associated with cancer. Unfortunately, a precise relationship between grades of epithelial dysplasia, carcinoma in situ, and invasive behavior has not been defined. Because malignant change has been associated with alterations of cell turnover and proliferation, we correlated histopathology with cell turnover studies in esophageal columnar epithelium from 11 patients. Biopsies from 8 of the 11 patients showed no dysplasia. Of the 2 patients with carcinoma, 1 had severe dysplasia and 1 had minimal change on the tissue sections studied. In addition, 1 patient without carcinoma had minimal dysplastic change.

In the esophageal columnar epithelium, cell kinetics studies were restricted to the proliferating compartment which corresponded to the crypt-like structures. The mean DNA synthesis phase of 10.4 hr was similar to values (10 to 13 hr) usually found in respiratory and intestinal epithelium of man using in vivo and in vitro techniques. Many tumors, including squamous cell carcinoma of the esophagus, have S phases in excess of 20 hr. The patients with cancer in this study did not have a prolonged S phase when compared to the other patients with columnar esophageal tissue. The LI of cells in the proliferative compartment (23.3%) was similar to values reported for small intestine, colon and rectum (10 to 25%), and the mean MI of 1.8% was similar to that of small intestinal crypt cells.

The LI, MI, and synthesis time of the basal cells of squamous esophageal tissue in 7 of our patients was similar to values previously reported. The synthesis phase of the basal cells was similar to the columnar tissue, but the LI, GT, and MI of the squamous epithelial compartment was approximately one-half that of the columnar epithelium. These data indicate that cell turnover of esophageal columnar tissue is more comparable to other columnar epithelium than to squamous esophageal tissue.

The most striking finding was the incorporation of tritiated thymidine into the nuclei of cells on the luminal surface from 2 patients proved to have definite adenocarcinoma in other areas of the esophagus. One patient without adenocarcinoma, A. H., also had surface cell labeling. Repeated cytological and histological studies failed to show carcinoma in this patient. In the biopsy sections studied by radioautography, mitotic figures were not observed in epithelial cells on the luminal surface. The absence of mitotic figures in the sections processed for radioautography does not indicate that cells at the luminal surface incorporating [3H]thymidine were not replicating. Only occasional surface cells incorporated [3H]thymidine into nuclear material and the ratio of labeled cells to mitotic figures was approximately 13 to 1. Thus one could anticipate that mitotic figures would be extremely rare and could be easily overlooked.

Surface cell incorporation of tritiated thymidine is of especial interest since Lipkin has demonstrated that one of the first changes associated with malignancy in the colon is the size of the proliferative compartment. Using an organ culture technique in which cells were exposed to [3H]thymidine for 3 hr, Pelish and co-workers found labeled surface cells in biopsies from 3 patients with columnar esophageal epithelium who did not have evidence of adenocarcinoma. The more extensive surface cell-labeling in these patients may be related to exposure time to [3H]thymidine, which was 6 times longer than our technique and may reflect DNA repair or synthesis initiated after biopsy. We detected surface cell labeling in 2 patients with adenocarcinoma and only 1 of 9 patients without evidence of cancer, using a brief exposure to [3H]thymidine. Obviously, more studies will be necessary in order to assess the clinical value of cell proliferation studies as a diagnostic aid in surveillance for malignant transformation of columnar esophageal epithelium.

REFERENCES