

CORRELATION OF EPITHELIAL PROLIFERATION AND SQUAMOUS ESOPHAGEAL HISTOLOGY IN 1185 BIOPSIES FROM LINXIAN, CHINA

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Epithelial proliferation is an active area of research in gastrointestinal cancer, but only a few studies have examined the relationship of esophageal epithelial proliferation and squamous histologic findings in populations with high rates of squamous esophageal cancer. In order to study this correlation, tritiated thymidine labeling was performed on 1185 esophageal biopsies from 745 residents of Linxian, China, a county with some of the highest esophageal-cancer rates in the world. Total labeling index (TLI = total labeled cells/total cells counted) was used to measure the amount of proliferation, and the proportion of labeled cells found in cell layers 4 to 10 (labeled cell fraction 4 plus, LF4+ = labeled cells in layers 4-10/total labeled cells) was used to measure the vertical distribution of proliferation. Of the biopsies, 979 were histologically normal, 51 showed acanthosis, 35 showed esophagitis, 116 showed squamous dysplasia, and 6 showed invasive squamous cancer. The mean values of both proliferation variables, stratified by histologic diagnosis, showed the following relationships: normal = acanthosis < esophagitis = dysplasia < cancer. The ranges of proliferation values overlapped extensively in all biopsy categories, so that measuring proliferation could not substitute for histologic diagnosis. It remains to be seen whether proliferation values, histologic diagnoses, or some combination of these methods is most predictive of subsequent esophageal cancer.

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Linxian is a rural county in northern China which has some of the world's highest incidence and mortality rates for esophageal cancer. In 1973-1975, the age-adjusted mortality rates in Linxian were 161/100,000 for men and 103/100,000 for women, and by age 75 the cumulative mortality from esophageal cancer was over 20% in both sexes (Li *et al.*, 1980; Li, 1982).

Abnormalities of epithelial proliferation have been proposed as an early step in gastrointestinal carcinogenesis (Lipkin, 1988; Fearon and Vogelstein, 1990). Only a few studies, however, have examined the proliferative activity of esophageal squamous epithelium in populations with high rates of squamous esophageal cancer (Muñoz *et al.*, 1985; Yang *et al.*, 1987; Wang *et al.*, 1990, 1993). Using tritiated thymidine as a marker, some (Yang *et al.*, 1987; Wang *et al.*, 1990, 1993) but not all (Muñoz *et al.*, 1985) of these studies have reported a correlation between proliferation variables and histologic findings in small numbers of biopsies. Two endoscopic surveys conducted in Linxian gave us an opportunity to evaluate this relationship in a much larger number of biopsies and a broader range of histologic diagnoses.

MATERIAL AND METHODS

Endoscopic surveys

The 2 endoscopic surveys were part of the nutrition intervention trials conducted jointly by the Cancer Institute of the Chinese Academy of Medical Sciences and the US National Cancer Institute (Li *et al.*, 1986). Informed consent was obtained from each subject prior to endoscopy. In 1985, 450 subjects were biopsied at standard sites in the middle and lower thirds of the esophagus. In 1987, a standard site in the middle third of the esophagus was sampled in 685 subjects. In

both surveys, the fresh 2.8-mm biopsies were incubated for 1 hr in tritiated thymidine solution (Eagle's basic salt solution with 10% FCS and 5 μ Ci of tritiated thymidine) at 37°C in a 95% oxygen atmosphere, fixed in 10% buffered formalin (1985 biopsies) or 95% ethanol (1987 biopsies), and processed into paraffin blocks. Adjacent sections from each block were used for autoradiography and routine histology.

Autoradiography

Autoradiography was performed as described (Wang *et al.*, 1990). A cell was considered labeled if at least 5 black grains were seen over the nucleus. Only the flat regions of epithelium between papillae were counted. An attempt was made to count 2000 cells in each biopsy in a segment 200 cells long and 10 cell layers thick, starting at the base of the epithelium. We counted 2000 cells in 78% of the biopsies, between 1000 and 2000 cells in 15%, and fewer than 1000 cells in 7%, due to technical limitations such as small biopsy size. The numbers of labeled and unlabeled cells in each cell layer were recorded.

Two proliferation parameters were derived from the cell-count data: the total labeling index (TLI = total labeled cells/total cells counted) was our measure of the total amount of proliferation; the proportion of labeled cells found in cell layers 4 through 10 (labeled cell fraction 4 plus, LF4+ = labeled cells in cell layers 4-10/total labeled cells) was our measure of the vertical distribution of proliferation in each biopsy.

Histologic categories

The histologic categories and criteria were as follows. Normal: a stratified squamous epithelium was present which showed no features diagnostic of acanthosis, esophagitis, squamous dysplasia or squamous cancer, as defined below. Acanthosis: an otherwise normal epithelium was ≥ 0.5 mm thick. Esophagitis: one or more of the following 3 criteria were present: both elongation of lamina propria papillae into the upper third of the epithelium and basal cell hyperplasia > 15% of the total epithelial thickness; epithelial infiltration by neutrophils or eosinophils; or a dense non-follicular infiltrate of mononuclear inflammatory cells or neutrophils in the lamina propria. Esophagitis was graded by the amount of inflammation present. Squamous dysplasia: nuclear atypia (enlargement, pleomorphism and hyperchromasia), loss of normal cell polarity and abnormal tissue maturation were present in the lower third (mild), the lower two thirds (moderate) or all thirds (severe) of the epithelium. Squamous cancer: malignant cells were present which had invaded through the basement membrane.

Analysis

After excluding biopsies with unsatisfactory histologic or radio-labeling data, the analysis was performed on 1185

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biopsies from 745 subjects (310 males and 435 females, with a mean age of 53 years and an age range of 33 to 71 years). For each proliferation parameter, the mean values of the biopsies in each histologic category were calculated using univariate analysis, correlated using Pearson correlations, and compared using the *t*-test procedure in the SAS statistical package (SAS Institute, 1989).

RESULTS

Table I shows the mean values of the proliferation variables stratified by histologic diagnosis. The pattern of results was similar for both variables. The average amount and vertical distribution of proliferation were similar in normal biopsies and in biopsies showing acanthosis ($p = 0.928$ for the difference in mean TLI values). Among the normal biopsies, a

sub-set showing basal-cell hyperplasia (without accompanying elongation of the lamina propria papillae) had higher proliferation values than the rest ($p < 0.001$). The mean proliferation values were similar in biopsies showing esophagitis and those showing dysplasia ($p = 0.625$), but these biopsies had higher mean values than those of normal or acanthotic mucosa ($p < 0.001$). Within the categories of esophagitis and dysplasia, proliferation values increased with increasing histologic grade. Biopsies showing invasive cancer had significantly more proliferation than those with dysplasia or esophagitis ($p = 0.005$). The overall pattern of results for both TLI and LF4+ was as follows:

normal = acanthosis < esophagitis = dysplasia < cancer

($r = 0.39, p < 0.001$ for TLI). This pattern was present both in the 1985 and in the 1987 biopsies, whether analyzed separately (data not shown) or combined.

Figure 1 shows the distribution of TLI values by histologic category. The ranges of the TLI values overlapped extensively in all diagnostic categories. A similar overlap was seen in LF4+ values (data not shown). Thus it was not possible to determine the histologic category of an individual biopsy from its radio-labeling data.

TABLE I - MEAN PROLIFERATION VALUES OF 1185 ESOPHAGEAL SQUAMOUS BIOPSIES FROM LINXIAN, CHINA, BY HISTOLOGIC DIAGNOSIS

Histologic diagnosis (N)	Mean TLI ¹ (%)	Mean LF4+ ² (%)
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DISCUSSION

Epithelial proliferation is an active area of research in gastrointestinal cancer (Lipkin, 1988). Only a few studies, however, have correlated proliferative activity and histologic findings in the esophageal squamous epithelium of individuals from populations with high rates of squamous esophageal cancer (Muñoz *et al.*, 1985; Yang *et al.*, 1987; Wang *et al.*, 1990, 1993). These previous studies have evaluated relatively few (29 to 147) biopsies and have included only limited spectra (2 to 4 categories) of histologic diagnoses. Endoscopic surveys performed during the nutrition intervention trials in Linxian, China, allowed us to examine this correlation in larger numbers of biopsies and a broader range of histologic categories.

¹Total labeling index. ²Fraction of labeled cells in cell layers 4 to 10. ³Basal-cell hyperplasia.

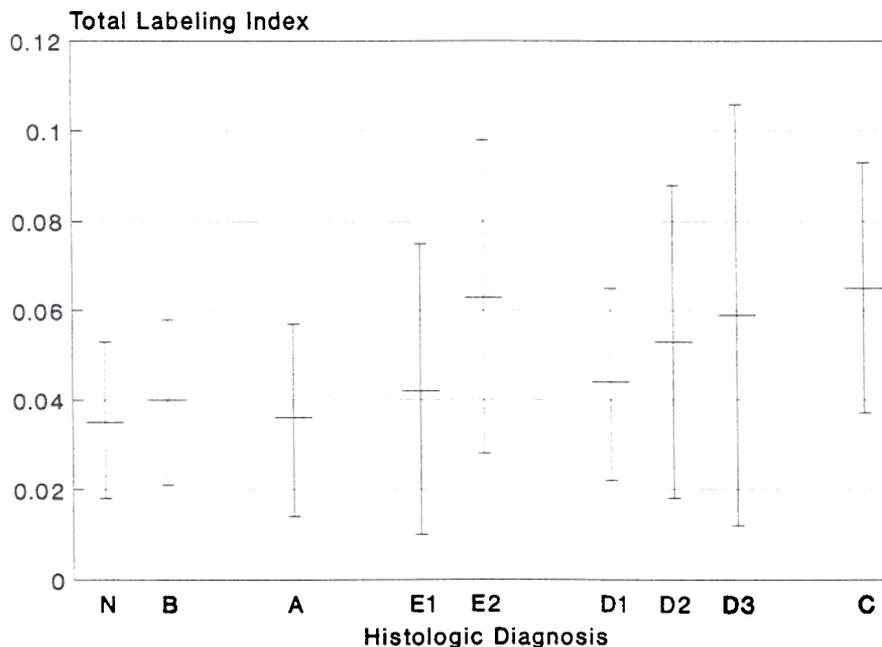


FIGURE 1 - Distribution of total labeling index (TLI), categorized by histologic diagnosis. N, normal without basal-cell hyperplasia; B, normal with basal-cell hyperplasia; A, acanthosis; E1, mild esophagitis; E2, moderate esophagitis; D1, mild dysplasia; D2, moderate dysplasia; D3, severe dysplasia; C, invasive cancer; —, mean; 1, 2 standard deviations.

Tritiated thymidine labeling can provide information about 2 aspects of cell proliferation: the amount and the site of proliferation. In our biopsies, we used the total labeling index (TLI) to measure the amount of proliferation and the proportion of labeled cells in cell layers 4 to 10 (LF4+) to measure the vertical distribution of proliferation.

Our data show a correlation between the mean values of both of our proliferation parameters and the histologic diagnoses, with similar patterns of correlation in the amount and vertical distribution of proliferation. These results agree with prior studies which have found more proliferation and more widely distributed proliferation in biopsies showing basal-cell hyperplasia, esophagitis and dysplasia than in biopsies of normal epithelium (Yang *et al.*, 1987; Wang *et al.*, 1990, 1993). Our results do not agree with the one study which showed similar labeling indices and a similar distribution of labeled cells in biopsies with and without esophagitis (Muñoz *et al.*, 1985). This latter study examined only 29 biopsies, 12 with and 17 without esophagitis, and this may have been too few to see differences in the proliferation results.

We classified acanthosis and basal-cell hyperplasia into separate histologic categories, unlike others who included both of these appearances in a single category of "hyperplasia" (Yang *et al.*, 1987). The distinction appears to have been appropriate, since the 2 groups had different proliferation values: the mean values of acanthotic biopsies were quite similar to those of normal biopsies not showing basal-cell hyperplasia, while the mean values of biopsies with basal-cell hyperplasia were more similar to those of biopsies with mild esophagitis or mild dysplasia. Wang *et al.* (1990, 1993) also

found the category of basal-cell hyperplasia to have a higher labeling index than normal biopsies. Livstone *et al.* (1977), while not categorizing biopsies by histology, found that the thymidine-labeling index increased with increasing thickness of the basal-cell layer.

While the results of our evaluation show an overall positive correlation between esophageal proliferation values and squamous epithelial histology, potentially important differences between these categorizations were also observed. For example, esophagitis and dysplasia had similar proliferation values but were histologically distinct. This difference in proliferation and histology results is especially interesting in the context of Linxian, where chronic esophagitis and dysplasia have been hypothesized to be related pre-cancerous lesions (Crespi *et al.*, 1979; Muñoz *et al.*, 1982). Work is in progress to determine whether proliferation results, histologic findings or some combination of the 2 are most predictive of subsequent squamous esophageal cancer.

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