Labeling Index and Labeling Distribution of Cells in Esophageal Epithelium of Individuals at Increased Risk for Esophageal Cancer in Huixian, China

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ABSTRACT

The pattern of proliferation of epithelial cells in esophageal epithelium was studied by means of [3H]deoxythymidine labeling of esophageal epithelium in subjects from Huixian, Henan Province, China, a high-risk geographical region for esophageal cancer. Comparisons were made among patterns of cell proliferation observed in normal esophagus, in hyperplasia, in mild dysplasia, and in moderate dysplasia in a total of 118 subjects. The amount of cell proliferation observed was lowest in normal esophageal epithelium and increased progressively in subjects having hyperplasia, mild dysplasia, and moderate dysplasia. The location of proliferating cells was limited mainly to the base of the esophageal epithelium in normal esophagus, but expanded toward the surface of the esophageal lining in individuals with hyperplasia and dysplasia. The larger total numbers of proliferating cells in the esophageal epithelium and the progressive expansion of the proliferative compartment toward the epithelial surface found in hyperplasia and in dysplasia could both facilitate the screening of subjects for esophageal cancer risk and serve as intermediate biomarkers in prophylactic dietary or pharmacological intervention studies.

INTRODUCTION

Because of the high incidence and mortality rate of esophageal cancer in certain countries, early detection and identification of their high-risk subjects are desirable. Previous studies from the counties of Linxian and Jixian in northern China had suggested that abnormal patterns of proliferation of epithelial cells in the esophageal lining were associated with increased levels of risk for esophageal cancer. One of the methods of detecting epithelial cell proliferation, [3H]dThd labeling, permits the numbers of S-phase cells distributed within the esophageal lining to be determined. By the use of that labeling technique hyperplasia and dysplasia had higher labeling index values than normal esophagus; and the proliferative zone in those precancerous esophageal diseases showed expansion toward the esophageal surface, in contrast to normal esophagus where that zone was confined to the lowest portion of the basal epithelium (1, 2). On the basis of these findings we studied proliferative patterns in esophageal epithelium in individuals from another high-risk county of China, Huixian, which is next to Linxian in the province of Henan, in both normal subjects and persons with hyperplasia, or mild or moderate dysplasias.

MATERIALS AND METHODS

In 1986, a mass survey with endoscopic examinations was performed in Huixian, Henan Province, People's Republic of China, a high-risk area for esophageal cancer. At every endoscopy, two biopsy samples were taken from the middle and lower thirds of the esophagus, and additional biopsies were taken from any macroscopic lesions that were observed. All the biopsies from the middle third of the esophagus were immediately incubated with tritiated thymidine (5 μCi/ml). Incubation was carried out in a 95% basal medium with 10% fetal calf serum (at the Institute of Henan Medical Sciences, Zhengzhou, People's Republic of China), using a shaking bath at 37°C for 1 h. The biopsies were then fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 5 μ. The slides were dipped in NTB-2 emulsion (Eastman Kodak Co., Rochester, NY), air dried, and exposed for 4 wk at 4°C. They were then developed in Kodak D-19 for 2 min, rinsed, fixed for 2 min, and rinsed again. The dried slides were stained with hematoxylin and eosin.

Five coded slides were prepared from each biopsy for each individual studied, and sectioned biopsy specimens from the esophageal lining were assayed in flat regions of the basal cell layer. The epithelial cells were counted in cell columns oriented perpendicularly to the basal cell layer, for the recording of [3H]dThd labeling versus cell position in the columns. In each cell column, cells were counted proceeding from the bottom of the basal layer toward the esophageal lumen. The detailed criteria for recording of labeled cells were the same as those previously described (1, 2).

Histopathologically, observations were made to record the presence of basal cell hyperplasia, mild dysplasia, moderate dysplasia, or normal epithelium, according to previous descriptions (2, 3). From the measurements of [3H]dThd cell labeling, statistics of labeling index values were computed by averaging those values with equal weights over the members of each group, by computing the corresponding standard deviation and standard error, and with unpaired t-tests.

RESULTS

Histopathology. According to the criteria described previously (2, 3), 19 cases with normal esophageal epithelium, 65 cases with basal cell hyperplasia, 10 cases with mild dysplasia, and 12 cases with moderate dysplasia were found and analyzed for [3H]dThd-labeling patterns.

Table 1 compares empirical observations made for the various groups studied: normals; basal cell hyperplasia; mild dysplasia; and moderate dysplasia. The data in Table 1 can be summarized as follows.

Number of [3H]dThd-labeled Epithelial Cells Found in Relation to the Number of Epithelial Cells Counted. In each of the abnormal groups studied, basal cell hyperplasia, mild dysplasia, and moderate dysplasia, the pooled number of epithelial cells found to be labeled was a larger proportion of the total number of epithelial cells than in the normal group. The proportions of epithelial cells labeled were larger by the factors 2.4 for basal cell hyperplasia, 2.4 for mild dysplasia, and 2.6 for moderate dysplasia, all significantly different (p < 0.001) from the normal group.

Cell Counts Analyzed by Epithelial Cell Layer. When cell counts pooled within groups were analyzed by cell layer, the number of labeled epithelial cells was again a significantly larger proportion of the total number of cells counted in the three disease groups studied, than in the normal group in most of the cell layers. This was found in Cell Layers 1 to 5 for the basal cell hyperplasia group and in Cell Layers 1 to 6 for the two dysplasia groups, with P < 0.001 in each case.

Labeling Index Statistics. The labeling index (fraction of
epithelial cells labeled) was computed by cell layer for each individual subject, and the elementary statistics (mean and standard error) of the resulting labeling index values were computed over each group of subjects, by cell layer. The mean labeling index was significantly larger than the normal group in Cell Layers 2 to 5 in the basal cell hyperplasia group (P < 0.001), in Cell Layers 2 to 4 in the mild dysplasia group (P < 0.001), and in Cell Layers 2 to 5 in the moderate dysplasia group (P < 0.001), and in Cell Layers 2 to 4 in the mild dysplasia group (P < 0.001), and in Cell Layers 2 to 5 in the moderate dysplasia group (P < 0.001). Thus, compared with the esophageal epithelium of the normal group, the basal cell hyperplasia group; and by factors of 2.4 and 2.6 in the mild dysplasia group. The lower dashed line shows the labeling index profile for the basal cell hyperplasia group; and the dotted line shows the profile for the esophageal lining of the normal group. The height of each rectangular bar plotted represents the mean ± SE.

In normal subjects, the esophageal epithelium had a distribution of labeled cells with maximum cell proliferation occurring in Basal Cell Layers 1 and 2 at the bottom of the epithelial lining; a marked decrease in cell proliferation was found above these cell layers (2). In hyperplasia and in mild and moderate dysplasia, maximum cell proliferation occurred in Cell Layer 2 with a 2-fold increase in labeling index; in addition the zone of cell proliferation expanded markedly in Cell Layers 3, 4, and 5. Thus Fig. 1 illustrates the progressive increase in cell proliferation and expansion of the proliferative compartment, occurring in the esophageal epithelium in precancerous diseases of increasing severity in individuals in Huixian.

**DISCUSSION**

Measuring proliferating cells by pulse labeling with [3H]dThd has been used previously to define proliferative characteristics...
of epithelial cells in precancerous esophageal diseases. The epithelial lining of the esophagus is readily accessible to biopsy, and labeling of cells in vitro was carried out in this study. Muñoz et al. reported a significant difference in the pattern of esophageal cell proliferation in a high-risk group for esophageal cancer compared with a low-risk group. In the high-risk group, cell proliferation occurred more often in the intermediate and superficial layers of the esophageal epithelium, suggesting that high labeling index values could represent a risk factor for esophageal cancer (1). Yang et al. (2) similarly showed a relationship of the amount of cell proliferation and risk for esophageal cancer. The results of this paper show that in 118 subjects studied in Huixian, Henan Province, an increase in the overall labeling index when normal esophageal epithelium progressed to hyperplasia, to mild dysplasia, and to moderate dysplasia.

Analysis of the distribution of proliferating cells within the esophageal epithelium has provided further information. In normal epithelium the zone of cell proliferation was located in the first two layers of basal epithelium. An expansion of the proliferative compartment toward the luminal surface of the epithelium was observed in the hyperplasia group and in mild dysplasia and moderate dysplasia. The labeling index profile was higher in the moderate dysplasia compared with the mild dysplasia group, although differences were not significant.

In conclusion, these findings further suggest that the increasing severity of precancerous esophageal diseases is associated with increased proliferation of the esophageal epithelial cells; abnormal differentiation of the cells is also currently under study. Findings support the assumption that an increased risk of cancer is linked to the degree of cell proliferation occurring in the esophagus, as occurs in other areas of the gastrointestinal tract (4). As these precancerous changes evolve in the esophageal epithelium of individuals in Huixian, etiological factors could include the presence of dietary genotoxic substances (5) that contribute to cell damage. Such substances could become tumor-initiating agents if damaged cellular DNA did not undergo adequate repair prior to cell proliferation, leading to altered DNA introduced into daughter cells that survived the damage. In the high esophageal cancer region in northern China, increased cell proliferation might also be influenced by micronutrient deficiencies in a cereal-based diet (6). Further replication of abnormal cells could then contribute to the evolution of neoplastic cells in a multistep process. Increased cell proliferation has been associated with tumor promotion in various experimental models (7,8). A recent review summarizes current data of gastrointestinal epithelial cell hyperproliferation, including the esophagus, and initiation and promotion events, with application of cell proliferation measurements to studies of cancer prevention (4).

Thus, the observed increase in total numbers of proliferating human esophageal epithelial cells and an expansion of the proliferating layer of cells from the bottom of the esophageal epithelium toward the esophageal lumen could function in the postrinitiation phases of tumor development, in particular during the phase of tumor promotion. The identification of these findings appears to be capable of aiding the definition of individuals who are at increased risk for development of esophageal cancer, suggesting that regular examinations for early detection of neoplastic cell transformation be carried out. The regularity with which these changes have now been found to occur (i.e., Refs. 1 and 2 and the present study) further suggests they can also serve as intermediate biomarkers to improve the definition of cellular changes occurring during prophylactic dietary or pharmacological intervention studies. Cell proliferation measured by the present pulse-labeling technique is being used as an intermediate biomarker in a large scale intervention trial on esophageal cancer in Linxian, China, using a combination of multiple micronutrients (9).

REFERENCES

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