Accumulation of p53 Protein in Human Esophageal Precancerous Lesions: A Possible Early Biomarker for Carcinogenesis

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ABSTRACT

The level of p53 protein was determined immunohistochemically in normal tissues and tissues with different severities of lesions (basal cell hyperplasia, dysplasia, carcinoma in situ, and carcinoma) from surgically resected human esophagi and esophageal biopsies of symptom-free subjects. The samples were from an area with high esophageal cancer incidence in northern China (Linxian and Huixian in the Henan province). Tissue sections were incubated with p53 antibodies for immunostaining. Conventional hematoxylin and eosin stain was also used. In surgically resected esophageal specimens, elevated p53 protein levels were found in the cell nuclei in tissues with precancerous and cancerous lesions. From basal cell hyperplasia to carcinoma in situ, the p53 immunostain-positive cells increased in number, and their distribution had roughly the same pattern as that of the proliferating cells. However, positive stain was not observed in the dividing basal cells of the normal epithelium of the surgically resected tissues. A similar pattern of immunostaining was observed in the abnormal tissues of the biopsy samples from the symptom-free subjects. An intriguing observation is that some p53 immunostain-positive cells were observed in 3 of 6 cases of histologically normal epithelia of biopsy samples. Only the papillary immunostaining pattern was observed in these three “normal” cases. Although the molecular basis for such positive stain remains to be investigated, it is possible that p53 protein accumulation occurs early in the pathogenesis of esophageal cancer and that p53 mutation is closely associated with the initiation of this cancer. The accumulation of p53 protein may be a promising early biomarker for identifying high-risk subjects for esophageal cancer.

INTRODUCTION

Carcinoma of the esophagus is a very common disease in Linxian and Huixian in Henan province in northern China (1–3). Although the etiology and natural history of esophageal cancer are not clear, results from previous studies by us and others suggest that malignant transformation of human esophageal mucosa is a multistage progressive process (1–6). An early indicator of abnormality in persons predisposed to esophageal SCC is an increased proliferation of the esophageal epithelial cells, morphologically manifested as BCH, dysplasia, and CIS; most or all of these conditions could be considered precancerous lesions of SCC (1–6). The concept of the precancerous nature of these lesions originated from prospective follow-up studies on subjects in high-incidence areas for esophageal cancer (2, 3), comparative studies in populations in high- and low-incidence areas for esophageal cancer (2, 7), and pathological studies on the epithelium adjacent to SCC (8). Knowledge of human esophageal precancerous lesions at the molecular level is of critical importance to our understanding of the etiology of this disease and to the identification of useful biomarkers for prevention studies. However, little information is available on the molecular alterations associated with these lesions.

Deletion or mutation of tumor suppressor genes may be a key event in tumorigenesis. Much recent attention has been focused on the p53 gene. The human p53 gene is located on chromosome 17p13 and encodes a nuclear protein which may be vital in the regulatory control of cell proliferation (9–12). Loss of wild-type p53 function is considered to be a key event in the induction of malignant transformation in many cancers (9). Some mutations in p53 may confer oncogenic properties to the protein (9, 13). Frequent mutation of the p53 gene at exons 5, 6, 7, and 8 have been found in a wide variety of human cancer, including esophageal SCC (14, 15). Mutant p53 proteins are more stable than the wild type and thus their accumulation can be detected immunohistochemically (13). The present study was undertaken to determine the accumulation of p53 protein in precancerous lesions of resected esophagi from SCC patients and symptom-free subjects from a high-risk population for esophageal cancer to gain insight into the possible involvement of p53 protein in the early stage of esophageal carcinogenesis.

MATERIALS AND METHODS

Tissue Collection and Processing. Forty-four surgically resected specimens were collected from patients with primary esophageal carcinoma in Linxian and Huixian. Of the 44 specimens examined, 31 were from males (30 to 67 years of age with a mean ± SD of 52 ± 5 years) and 13 were from females (45 to 66 years of age with a mean of 56 ± 6 years). The patients had not received either radiation therapy or chemotherapy before surgery. The resected esophageal specimens, with an average length of 8 cm, were cut longitudinally and flattened. From these 44 specimens, samples of 1 cm² each were taken from different sites: visible tumor; normal mucosa adjacent to the tumor (transitional mucosa); and the end of the sample (Fig. 1). Of 132 samples, 65 were analyzed. Esophageal biopsies were also collected from 51 symptom-free subjects during a mass survey in Huixian. Of the 51 biopsies from the symptom-free subjects, 29 were from males (22 to 66 years of age with a mean of 44 ± 9 years) and 22 were from females (27 to 66 years of age with a mean of 43 ± 11 years). All of the tissues were fixed in 10% alcohol and embedded in paraffin. Each block contained one piece of tissue and was serially sectioned at 5 µm. The sections were mounted onto histostick-coated slides. Three or four adjacent ribbons were collected for histopathological analysis (hematoxylin and eosin stain) and for immunohistochemical staining.

Histopathological Analysis. Histopathological diagnoses were made according to the cellular morphological changes and tissue architecture using previously established criteria (2, 16). The epithelium was graded as “normal” for nonkeratinized stratified squamous epithelium with 1–3 basal cell layers; the papillae were confined to the lower half of the epithelium. In BCH, the number of proliferating basal cells was increased to more than three cell layers (2). Dysplasia was characterized by the partial loss of cell polarity and nuclear atypia. In CIS the esophageal mucosa was totally replaced by dysplastic epithelial cells but the basement membrane was still intact. SCC was characterized by confluent and invasive sheets of cohesive, polymorphous cells with hyperchromatic nuclei. Abnormal mitoses were observed in many samples.

Histopathologically, CIS was usually found adjacent to the SCC, and BCH and dysplasia were frequently observed as isolated lesions in the surgically resected esophageal specimens. Of the 65 samples examined, 14 were diag-
esophageal lesions from normal to BCH to dysplasia to CIS, the cell proliferative activity. The positive cells were distributed in the lesions. Although other interpretations are possible, the observed accumulation of p53 protein immunostaining pattern was also found in the different lesions. In tissues with clearly distinguishable areas of normal and abnormal mucosa, the p53 positively stained cells were found in the abnormal mucosa but not in the adjacent normal mucosa (Fig. 2C). The mature cells in the middle and superficial layers of the esophagus and stroma cells always stained negatively (Fig. 2D).

A similar pattern of p53 protein immunostaining was also observed in biopsy samples from symptom-free subjects (Fig. 3). The biopsy samples usually contained only epithelial cells. Immunostain-positive cells were observed in dysplasia and BCH and along the longitudinally sectioned papillae or around the cross-sectioned papillae (Fig. 3, A and B). An unexpected finding in these biopsy samples was the observation of p53 positive stain cells in normal esophageal epithelium (Fig. 3, C and D). Of the 6 apparently normal esophageal epithelial biopsies from the symptom-free subjects identified by histopathology, 3 cases (2 males aged 44 and 53, one female aged 30) showed positive p53 immunostaining; only a papillary immunostaining pattern was observed in these three cases. In a control experiment for Fig. 3D, in which the antibody to p53 was omitted from the immunostaining procedure, positively stained cells were not observed (Fig. 3E). No clear-cut positively immunostaining cells were found in the other 3 apparently normal biopsy samples and the 14 normal epithelial samples from the surgically resected tissues. These staining patterns were confirmed by another experiment in which 6 normal samples from biopsy and 6 normal samples from resected tissues were immunostained side by side.

A “diffuse” immunostaining pattern was frequently observed in SCC, CIS, and dysplasia (Fig. 2, A–C). Of the 5 dysplastic epithelia from the symptom-free subjects, 3 cases showed the “diffuse” immunostaining type (60%; Fig. 3A). The “papillary” immunostaining pattern was usually observed in the less severe lesions (Fig. 3, C and D). Both “focal” and “scattered” patterns were observed in the precancerous and cancerous lesions.

The relationship among p53 immunostaining patterns and histopathology was shown in Table 1. Positive stain rates of more than 78% were found in all precancerous and cancerous tissues. With the lesion progression from BCH to dysplasia to CIS and SCC, the incidence of diffuse pattern increased and reached its highest point with SCC (78%). The incidence of papillary pattern was higher in the biopsy sample (29 of 51 biopsy samples) than in resected specimens (one of 65 samples). The reason for this difference is not known and is being investigated.

**DISCUSSION**

This study shows that p53 protein accumulated with increasing frequencies in the proliferating cells in BCH, dysplasia, CIS, and SCC. With the total 95 samples examined, positive p53 protein immunoreactivity was always localized in the cell nuclei. The observation that a “diffuse” immunostaining pattern occurred more frequently in more severe lesions whereas a “papillary” pattern was observed more frequently in the less severe lesions suggests the increased accumulation of p53 protein with the progression of the lesions. The failure to detect p53 positively immunostained cells in some precancerous or cancerous lesions suggests that p53 alteration is not an obligatory process in esophageal carcinogenesis.

Although other interpretations are possible, the observed accumulation of p53 protein is likely to be due to the mutation of the p53 gene. The mutant p53 proteins are more stable. Even in the presence of the wild-type p53 proteins, mutant p53 protein may render them in a mutant conformation through the formation of mixed dimers or oligomers.
Fig. 2. p53 immunostaining of surgically resected esophageal cancer and adjacent cancer tissues. Immunoreactivity is located in the nuclei of proliferative cells in: A. SCC; B. CIS; C. CIS (right), dysplasia (middle), and normal (left); D. BCH. No immunoreactivity was observed in E. normal tissue, and in the stroma cells. A'–E'. H&E stains of serial sections corresponding to Fig. A–E. ×270.
Fig. 3. p53 immunostaining of esophageal biopsies from the symptom-free subjects. Immunoreactivity is located in the nuclei of proliferative cells in: A, dysplasia; B, BCH; and C, normal. ×270. D, immunostain of the normal epithelium; ×1080. E, negative control of D, in which the antibody to p53 was omitted from the immunostaining procedure. A'–D' are H&E stains of sections corresponding to A–D.
tetramers (13). Bennett et al. (19) reported that in a subset of 10 human esophageal cancer samples, missense point mutations were found in 4 of 5 p53 immunostain-positive samples, whereas the wild-type p53 sequence was found in 4 of 5 immunostain-negative tumors. Analysis of preinvasive dysplasia or CIS adjacent to surgically resected tumors from 4 patients with SCC revealed that all of these lesions contained excess p53 protein by immunohistochemistry, and different mutations in the p53 gene were found in dysplasia (codon 272, GTG→TGTG) and invasive tumors (codon 282, CGG→TGG) from one patient (20). It has been reported that in human lung tumors (21) and colorectal cancer cell lines (22), the immunochromically detected p53 proteins were of the mutant type. On the other hand, p53 mutation in esophageal hyperplasia, a state known to revert back to normal, is a rather unexpected event. At the present time, however, we cannot exclude the possibility that the currently observed accumulation of p53 protein is due to an altered state of phosphorylation of the p53 protein, the stabilization of p53 proteins through binding to unknown factors, or an unusual overexpression of the wild-type p53 protein.

It is worth noting that in the biopsy samples, almost all of the BCH samples (39 of 40) were immunostained positive. The age distribution of these subjects was similar to that of the subjects biopsied. In the biopsy sample with normal epithelia and BCH, p53 positive cells were frequently observed in the papillary regions. This finding suggests that basal cells within the regions of the papillae may be the earliest site for carcinogenesis. Yang and Lipkin (23) reported that the papillae were more intensely stained than other areas with AE1 cytokeratin, a proposed biomarker for identifying abnormal cell proliferation. It has been reported that elongation of papillae was more frequent in subjects in high-risk areas for esophageal cancer than those in low-risk areas (2). Young persons (15 to 26 years of age) from households with esophageal cancer patients had a higher incidence of superficial elongation of the papillae than those from noncancer households (24). The “papillary” immunostaining pattern of p53 may prove to be a sensitive pathological parameter for evaluation of the epithelial abnormality. Although the molecular basis for the existence of p53 immunostain-positive cells in the histologically normal epithelia of biopsy samples is not known, this may reflect a very early event in esophageal carcinogenesis. Additional studies with more subjects and follow-up studies on their further development of precancerous lesions and cancer would be of great importance.

The present results raise an interesting possibility that p53 protein accumulation, possibly due to p53 gene mutation, is a very early event in human esophageal carcinogenesis. A recent report also suggests that the accumulation of p53 protein is an early event in the development of human squamous cell carcinoma of the larynx (25). More additional work is needed to further substantiate the role of p53 alterations in early lesions of the esophagus. Such an effort is of vital importance not only for the understanding of molecular mechanisms of esophageal carcinogenesis but for the development of an early biomarker for prevention studies.

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