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A preliminary study on ras protein expression in human esophageal cancer and precancerous lesions

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INTRODUCTION

The esophageal carcinoma is a common malignant tumor in Linzhou City (Linxian) of Henan Province in northern China. Although the etiology and natural history of esophageal carcinoma are not clear, a substantial amount of evidence has been provided to suggest that the development of human esophageal squamous cell carcinomas (SCC) is a multistage progressive process[1-4]. An early indicator of abnormality in persons predisposed to esophageal SCC is an increased proliferation of esophageal epithelial cells, morphologically manifested as basal cell hyperplasia (BCH), and dysplasia (DYS), and carcinoma *in situ*, which could be considered precancerous lesions of esophageal SCC[1-4]. Current molecular biology has suggested that many genes could participate in the different stages in esophageal carcinogenesis, and the synthetic effect of these different molecules might result in the malignant transformation of human esophageal mucosa[5,6], e.g., the amplification and/or overexpression of certain gene such as hst-1, int-2 and c-myc, have been observed in esophageal tumor s, moreover, the alteration of tumor suppressor gene p53 is one of the important molecular changes in the early stage of esophageal carcinogenesis[2-6].

Recent evidence has suggested that oncogene ras may play a role in cell growth and differentiation[7,8]. The p21 protein encoded by the ras gene family functions as G protein that participates in membrane signal transduction pathways[7]. Some sites of point mutation of ras oncogene, especially codons 12, 13, and 61, could be involved in malignant transformation cells[7]. Several reports have suggested a close association between specific ras gene mutation patterns and suspected etiological factors in some human cancers, for example, N-ras mutations have been associated with melanomas induced by sunlight[7]. ras gene mutations have been found in a significant percentage of human tumors, e.g. 75%-93% in pancreas adenocarcinoma, 7%-44% in colon cancer[7]. No ras point mutations have been found in DNA extracted from primary human esophageal tumors in France and South Africa, this is in sharp contrast to some reports in which rat esophageal papillomas were induced by *methylbenzyl*-nitrosamine[8]. This prompted us to re-evaluate the role of the ras family of oncogene in human esophageal carcinogenesis. In the present study, we analyzed the expression of ras protein in cancerous and precancerous tissues of the esophagues collected from the subjects in Linzhou, a high incidence area of esophageal carcinoma in China.

MATERIALS AND METHODS

Tissue collection and processing

Esophageal biopsies were taken from 54 symptom-free patients who volunteered to participate in a routine endoscopic screening for esophageal carcinoma in Linzhou City, China. Of the 54 subjects examined, there were 29 males (30-72 years of age, with a mean±SD of 47±15 years) and 25 females (32-70 years of age, with a mean±SD of 44±17 years). The surgically resected specimens were obtained from patients (36 patients, aged 40 to 74 years, with a mean±SD of 55±10 years) with primary esophageal carcinoma in Linzhou City, China. The patients had received no radiation therapy or chemotherapy before surgery. All of the tissues were fixed in 80% alcohol and embedded in paraffin. The section was 5 μm in thickness. Three or four adjacent ribbons were collected for histopathological analysis (hematoxylin and eosin stain), and for immunohistochemical staining.

Histopathological analysis

Histopathological diagnoses for esophageal epithelia were made according to the cellular morphologic

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changes and tissue architecture using previously established criteria[1-4]. In brief, the normal esophageal epithelium contained one to three proliferating 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; DYS was characterized by nuclear atypia (enlargement, pleomorphism, and hyperchromasia), loss of normal cell polarity, and abnormal tissue maturation. SCC was characterized by confluent and invasive sheets of cohesive, polymorphous cells with hyperchromatic nuclei.

Antibodies and reagents

The monoclonal antibodies (Pan-ras) Ab-3 (Oncogene Science Inc, USA) were mouse antibodies against human ras protein. Twenty mL/L crystalline bovine serum albumin (BSA), (Sigma Chemical Inc, USA) and ABC and DAB kits (Vector Laboratories Inc. USA) were used for immunohistochemical assay.

Immunohistochemical staining

The avidin-biotin-peroxidase complex (ABC) method was used for ras protein. Briefly, after dewaxing, inactivating endogeneous peroxidase activity, and bloc king cross-reactivity with normal serum, the sectins were incubated overnight at 4 ℃ with a diluted solution of the primary antibodies (1:100 for ras). Locatio ns of the primary antibodies were achieved by subsequent application of a biotinylated anti-primary antibody, an avidin-biotin-complex conjugated to horseradish peroxidase, and diaminobenzidine. Normal serum blocking and omission of the primary antibody were used as negative controls.

The positive reaction was graded according to the number of brown-yellow particles appearing in cytoplasm. The diagnostic criteria for immunoreaction was based on previous reports[2-4].

RESULTS

Histopathological analysis

Histopathologically, among 54 esophageal biopsies, 12 had normal esophageal epithelium (22%), 34 had BCH (63%), and 8 had DYS (15%). All of 36 surgically resected specimens were found to have esophageal invasive SCC.

Immunohistochemical analysis

By immunohistochemical analysis, immunostaining of ras protein was predominantly observed in cytoplasm. The positive immunostaining of ras protein was not found in the normal tissues and the tissues with different severity of lesions from esophageal procancerous biopsies. The immunoreactivity of ras protein was observed in 9 (25%) of the 36 surgically resected esophageal cancer specimens (Table 1).

Table 1 Expression of ras protein in different severity of lesions from esophageal biopsies and carcinomas

Histological types	*n*	ras positive immunoreaction *n*(%)
Normal epithelium	12	0 (0)
Basal cell hyperplasia	34	0 (0)
Dysplasia	8	0 (0)
Esophageal carcinoma	36	9 (25)
Total	90	9 (10)

DISCUSSION

The unique observation in this study is that, the expression of ras protein was not observed in the normal esophageal epithelial and precancerous tissues, and the immunoreactivity of ras protein was observed in 9 (25%) of the 36 surgically resected esophageal cancer specimens. These results suggested that the expression of ras protein only occurs in the late stage of esophageal cancers, but not in early stage, and it might not be the important molecular change in the carcinogenesis of esophageal squamous epithelium.

The mutation of ras family of genes occurs extensively in human tumors, particularly tumors of the gastrointestinal tract and lung, the incidence being about 40% in colon cancers. Many researchers agree with the concept that the mutation of oncogene ras plays an important role in carcinogenesis of colon cancers[7,8]. However, the conclusions of oncogene ras in esophageal carcinogenesis were not consistent. Hollstein *et al*[9] found that tumor suppressor gene p53 had G-T transversion of mutation pattern in lesions of esophageal cancer in the areas where the tumor is closely associated with tobacco consumption, but the mutation of oncogene ras was not found in the same areas. No ras mutation was found with PCR technique in other areas of the world with high incidences of esophageal SCC, such as China[10] or South Africa[11], where N-nitrosamines and fungal toxins could play major roles. Experimentally, activating point mutation at codon 12 of H-ras was reported in rat esophageal tumor (papil lomas) induced by the carcinogen methylbenzyl-nitrosamine[12]. This was a typical example that the results of human molecular research was obviously unsimilar to animal studies. On the other hand, two recent studies reported that genomic amplification rates of K- and H-ras gene were up to 14% (7/51) and 40% (4/10) of esophageal cancer, respectively from France[13] and Canada[14], and one

immunohistochemical study of esophageal SCC from Italy and Amerca showed that the positive staining of the ras protein was 88.5% (46/52)[15]. Our results in the present study suggested that in the normal tissues and the tissues with different severity of lesions from esophageal biopsies, the expression of ras protein was not detected. Ras protein immunoreactivity was observed in 9 of the 36 surgically resected esophageal cancer specimens, accounting for 25%. The different results from the world suggested that the environmental factor might play an important role in esophageal carcinogenesis. Obviously, comparison with the molecular changes in the similar tumors in populations from different areas, is of great importance to reveal its carcinogenesis and further to understand its related etiology.

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