Overexpression of P53 and its risk factors in esophageal cancer in urban areas of Xi'an

QIAO Gui-Bin[1], HAN Cheng-Long[2], JIANG Ren-Chao[1], SUN Chang-Sheng[3], WANG Yan[3] and WANG Yun-Jie[3]

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Abstract

AIM To investigate the risk factors of esophageal cancer (EC) in urban areas of Xi'an and to determine the association between overexpression of P53 and these risk factors.

METHODS All cases (89) and controls (97) were permanent residents in urban areas of Xi'an, all cases of primary EC had been histologically confirmed, controls were inpatients with non-cancer and nonsmoking-related disease. Cancer tissues and tissues adjacent to the cancer of 65 cases and 24 available normal esophageal tissues of controls were detected for P53 overexpression by the immunohistochemical method.

RESULTS The smoking and familial history of cancer were significantly associated with EC in Xi'an inhabitants. The laboratory assay indicated that P53 positive stain in EC was 50.0%(34/65)and 6.1%(4/65) in tissues adjacent to the cancer, but no positive stain was found in normal esophageal tissues of controls. The results showed that P53 overexpression in EC was closely related to smoking and cases with familial history of cancer.

CONCLUSION Smoking and familial cancer history were important risk factors for EC,and the alteration of P53 gene may be due to smoking and inheritance factors.

[1]Department of Thoracic and Cardiovascular Surgery, Guangzhou General Hospital of Guangzhou Military Command, PLA, Guangzhou 510010, China

[2]Laboratory of Cellular Oncology, National Cancer Institute, USA

[3]Department of Epidemiology, the Fourth Military Medical University, Xi'an

Dr. QIAO Gui-Bin, male, born on 1969-07-20 in Taiyuan, Shanxi Province, graduated from the Fourth Military Medical University, as a postgraduate in 1996, specialized in research and treatment of thoracic diseases, having more than 10 papers and 3 books published. Tel. +86·20·86694772

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Correspondence to Dr. QIAO Gui-Bin, Department of Thoracic and Cardiovascular Surgery, Guangzhou General Hospital of Guangzhou Military Command, PLA, Guangzhou 510010, China

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INTRODUCTION

Esophageal cancer (EC) is the sixth most common cancer in males. In some areas of China, it is the leading cause of deaths. Previous studies suggested that certain local dietary and environmental factors are important risk factors in the high-incidence areas of China. But in Europe, epidemiological studies have shown that tobacco and alcohol consumption are the prevailing risk factors[1].

Recent progress in the molecular biology of cancer has a provided a basis for novel strategies in the prevention and treatment of cancer[2]. Cumulating evidence indicates that changes in both dominant oncogene and tumor suppressor genes are likely for malignant transformation of normal cells. Among these genetic abnormalities, the p53 tumor suppressor gene appears to be the most frequent target for DNA damage in carcinogenesis. Recent work suggests that proper function of this gene product in controlling growth is compromised by mutations through a significant region of the coding sequence[3], however, the exact cause of p53 gene mutations is still not clear until now. The current study was initiated to find out the risk factors of esophageal cancer in Xi' an inhabitants, and to correlate p53 overexpression with these factors.

Because almost all of previous studies in etiology of esophageal cancer were limited to study in the high-incidence areas of esophageal cancer, and in general, the high-incidence area tended to be rural[9], the results of these studies could not explain completely the etiology of esophageal cancer in Xi' an.

MATERIALS AND METHODS

Study design and inclusion criteria

The hospital-based case-control study was performed between August 1994 to January 1995. The subjects were selected from one of the three hospitals affiliated to the Fourth Military Medical University in Xi' an. All eligible subjects should be the permanent residents of the urban area of Xi' an. Informed consent was obtained from the subjects, if they agreed with the immunohistochemical and genetic analysis of tissue samples.

Our cases were selected from among consecutive persons who underwent major esophageal surgery and endoscopy, all were histologically confirmed as primary carcinoma of esophagus and were diagnosed for the first time, none of the case had chemotherapy or

radiotherapy prior to biopsies. Patients with esophageal cancer were excluded if they had a clinically apparent malignancy of any other origin.

Controls had a variety of diseases excluding cancer and diseases which are epidemiologically unrelated to tobacco smoking and aged from 45 to 65 years. Other malignancies were excluded by medical history and routine examinations.

Factors investigated

Factors in the questionnaire included demographic information (such as race, age, sex, educational level, and profession), tobacco smoking, alcohol consumption, intake of pickled vegetables, familial history of cancers, etc. All cases and controls were investigated by two trained investigators and interviewed for 20 minutes.

Tobacco smoking. Data on smoking history, i.e., duration of smoking, starting age, time of quitting, smoking, and average number of cigarettes consumed per day, were recorded.

Family history of cancer. Positive family history of cancer was defined as the presence of esophageal cancer in one or more relatives for both cases and controls. Data of three degree blood relatives on sex, age (age of death), cause of death, and duration of blood relatives living together with the subjects were investigated.

Samples

The tumor tissues were obtained from surgical or endoscopic resections. Nonpathologic or grossly normal esophageal tissues were obtained from the margins of the specimen or normal esophageal tissues apart from tumors under endoscopy. Tissue blocks of both grossly apparent carcinoma had nonlesional esophagus were fixed in 4% paraformaldehyde in standard phosphate-buffered saline (PBS), pH 7.4. They were irradiated with microwaves for 10-15 seconds and postfixed for 2 hours at 4℃ and then embedded in paraffin.

Immunohistochemistry

Thin 7 μm slices from paraffin-embedded specimens were deparaffinized routinely, then, added 50 μl 0.3% hydrogen peroxide for 10 minutes, followed by wash in PBS (pH 7.4) three times and treated with 50 μl 1% normal rabbit serum for 10 minutes at room temperature. The slices were incubated with primary antibody for 3 hours at room temperature in a moist chamber. The primary antibody used in this study was monoclonal antibody DO-7, purchased from Maxim Biotech Inc, China. The antibody reacted with wild and mutant type of the P53 protein. Immunostaining was performed by the streptavid in peroxidase conjugated method employing DAB as chromogens[4]. Specific staining was identified by the presence of brown or yellow reaction products.

Validity of laboratory measurement

All immunohistochemical studies were done without knowing the clinical data. To determine whether the positive stain resulted from contamination, two kinds of negative controls were used. Sections were incubated with PBS and non-immune mouse serum instead of the primary antibody. All sections for immunohistochemical analysis were observed by two experienced pathologists and their judgements should be in close agreement.

Statistical analysis

The odds ratio(OR) and its 95% confidence interval (CI) were calculated for variables that had been proved statistically significant in univariate analysis. Univariate analysis of factors potentially related to esophageal cancer and P53 overexpression were made to investigate the single effect of these factors. Logistic regression analysis was also performed. Univariate and multivariate analyses were conducted with SPLM program provided by the Department of Statistics in our university.

RESULTS

Subjects

Eighty-nine cases and 97 controls were collected. Their mean age (55.2 ± 9.7 years among cases and 54.4 ± 8.8 years among controls), proportion of gender, educational level and profession were similar. Detailed information on smoking and family cancer history was collected from all of our cases and controls.

The risk factors of esophageal cancer

The results indicated that the proportion of smokers and those with family histories of esophageal cancer was significantly higher than that in controls. It suggested that smoking and family cancer history were risk factors of EC. The OR for smokers was 3.26(95% CI 1.74 - 6.12), and for individuals with positive family cancer histories was 10.48(95% CI 4.81 - 22.48) (Table 1). Multiple logistic regression analysis showed the same results, in the logistic model, the interaction term was not statistically significant (probably due to sample size limitation) (Table 2).

Table 1 Single variable analysis of risk factors of EC

Factors	Cases	Controls	χ^2	OR	95% CI*	*P*
Smoking						
Yes	65	44				
No	24	53	15.53	3.26	1.74-6.12	0.0012
Family history of cancer						
Yes 43 8						
No	46	83	35.46	10.48	4.81-22.48	0.0000

*CI: confidence interval

Table 2 Logistic regression analysis of risk factors of EC

Factors	OR	Standard error	u value	*P*
Smoking	3.3	0.2939	4.0760	0.0000
Family history of cancer	3.0	0.2922	3.7090	0.0002

Table 3 Correlation between clinical features and overexpression of p53 in EC

Clinical features	P53 status		Univariate		Multivariate	
	Overexpression	Normal	χ^2	*P*	OR	*P*
Age	55.41±8.83*	55.32±11.07*	1.5836	0.9710	1.0104	0.8447
Sex						
Male	20	22				
Female	14	9	1.1497	0.5628	1.3611	0.7820
Smoking						
Yes	30	20				
No	4	11	3.8898	0.0486	3.1200	0.0041
Family history of cancer						
Yes	24	7				
No	10	24	17.2834	0.0001	15.8100	0.0002

*mean ± SD.

Immunohistochemical analysis

All cases, which were immunohistochemically detected, were squamous cell carcinomas. Among the 65 cases of esophageal cancer 52.0%(34/65) were positive for P53, 31 cases were negative for the immunostain. 6.1% (4/65) tissues adjacent to the carcinoma were positive for P53, but no positive products were observed in normal esophageal tissues of 24 controls. In the majority of tumor cells intense P53 immunostaining was observed in the cell nuclein and some tumor cells demonstrated the intense staining in cytoplasm. In normal esophageal mucosa, immunoreactivity of P53 was observed sporadically in basal and parabasal cells of mucosa. As a histological feature, the overexpression of p53 gene had heterogeneity in esophageal carcinoma. Immunoreactivity described earlier was not observed when incubated with PBS or non-immune mouse serum instead of the primary antibody.

Correlation of overexpression of P53 with clinical data

This study was unblinded after completing detection of overexpression of P53. Statistical analysis for the overexpression of P53 was then performed to correlate with clinical data. We could not find any statistically significant association between the overexpression of P53 and age, gender and occupation. In univariate analysis, the smoking and family histories of cancer were closely related with the overexpression of P53 ($P < 0.05$). Using the logistic regression model, multivariate analysis was performed to investigate the independent risk of smoking or family history of cancer as a predictive factor for overexpression of P53. Smoking and familial history of cancer were the factors shown to be closely related to overexpression of P53 after adjusting for other covariates ($P<0.01$), e.g., the odds ratio for overexpression of P53 to develop in smokers can be estimated at 3-fold increase over nonsmokers. The odds ratio for individuals who had family cancer histories was 15.8 (Table 3).

DISCUSSION

Numerous epidemiologic studies in China have shown that both particular environmental exposure and certain local dietary factors were responsible for the carcinogenesis of esophagus, but almost all previous studies were limited to the high-incidence areas of EC. Generally, the high-incidence areas have tended to be rural, so the results of these studies can not explain completely the rationale of EC in Xi'an which is not a high-incidence area in China. Our findings contradict the previous studies which stated that there was nosignificant association between smoking and esophageal cancer in China, and it was found that smoking and family history of cancer were important risk factors of EC.

More and more researches have shown the tumor is a molecular disease. Carcinogenesis depends on many genetic events, including mutation of oncogene or/and tumor suppressor gene. Carcinogens with significant mutagenic activity are components of various substances associated with tobacco smoking. P53 tumor supressor gene mutation has been observed in esophageal tumor cells induced by *N*-methyl-*N*-benzylnitrosamine treatment which is one of components in tobacco[5].

It is now clear that the p53 gene is the most frequent target gene among the known genetic alterations in EC[6]. P53 protein is a 53 KDa nuclear phosphoprotein and is expressed by many normal cells[7]. A decade ago, it was found that P53 protein was specifically overexpressed in transformed cells and undetectable in normal cells[8]. Numerous studies have confirmed these observations and shown that P53 protein accumulation is a consequence of its stabilization[9]. Normal P53 protein is known to be rapidly eliminated due to its short half-life, therefore, in normal cells, intracellular levels of P53 protein are low, and accumulation of P53 protein is usually the result of which modifies the conformation and stability of the protein[9,10]. It is easy to analyse the expression of P53 protein with immunohistochemistry on a large panel of tumors, because there appears to be a good correlation between the p53 gene mutation and its protein accumulation. In accordance with previous studies, P53 protein accumulation was found in 50% tumor specimens and 6.1% (4/65) tissues adjacent to cancer. In our study, in order to investigate whether the overexpression of P53 was present in normal esophageal tissues of controls, we immunostained 24 available specimens of controls, but no positive staining was found. The results suggest that the overexpression of p53 gene is a frequent genetic alteration and plays an important role in the carcinogenes is of esophageal carcinoma.

At present, the P53 tumor suppressor gene has come to the forefront of cancer research because it is commonly mutated in human cancer and the spectrum of p53 mutations in these cancers can provide clues to the etiology[11,12]. Various studies indicate that the p53 gene is a good target for molecular epide miological studies of various human cancer. Our epidemiological investigation also showed that major fraction of EC can be attributed to smoking and familial history of cancer, therefore, identifying the association of P53 overexpression with smoking and family history of cancer in this study is of particular interest. The study showed that the alteration of p53 gene was associated with smoking and family history of cancer.

Because p53 genes are inherited in a recessive form, requiring the loss of both copies for the phenotype to be expressed, it can be hypothesized that genetic predisposition to cancer induction may be related to inherited mutations in all ele of tumor suppressor genes that regulate cell growth and terminal differentiation, and this kind of inherited susceptibility to esophageal cancer can be provoked by the specific carcinogens contained in cigarettes. Inactivation or altered function of these genes results in increased risk for development of tumors. The results of this study may be a dramatic example of this process, suscep tible hosts who inherit one defective allele of p53 gene can become patients when another allele is lost through later somatic mutation induced by carcinogens in cigarettes.

As stated above, family cancer history depends on many factors, such as the number of relatives, their biologic relationship to the index case, their age distribution and the disease frequency in the population, so it can not be concluded that the positive familial history of cancer is due to genetic susceptibility, and several types of mutation do not lead to P53 accumulation and would be missed by immunohistochemistry[13], therefore, our further study will be focusing on a larger sample collection, PCR-SSCP analysis and DNA sequencing, and the application of more efficient statistical methods.

REFERENCES

1 Yu MC, Garabrant DH, Peters JM, Mack TM. Tobacco, alcohol, diet, occupation and carcinoma of the esophagus. *Cancer Res*, 1988;49(18):3843 - 3848
2 Minna J, Nou M, Takahashi T, Shutte J, Chiba I, Viallet J *et al*. A molecular pathogenesis of lung cancer. In: Bergsageal DE, Mak TW, eds. Molecular mechanisms and their clinical applications in malignancies. ed 1. Orlando, FL: *Academic Press*,1990:63 - 83
3 Chiba I, Takahashi T, Nau M, Damico D, Curriel D, Mitsudomi T *et al*.Mutation in the p53 gene are frequent in primary, resected non-small cell lung cancer. *Oncogene*,1990;5(8):1603 - 1610
4 Zuorong, Shi. A comparison of three immunoperoxidase technique for antigen detection in colorectal carcinoma tissues. *J Histochem Cytochem*, 1988;96(2): 371 - 372
5 Guo Y, Lu S, Liu Y. Overexpression of p53 gene in esophageal cancer induced by nitrosamine.*Chin J Tumor*, 1992;14(1):241 - 244
6 Baker SJ, Fearon ER, Nigro IM, Hamilton SR, Preisinger AG, Jessup JM *et al*. Chromosome 17 deletion and p53 gene mutations in colorectal carcinomas. *Science*,1989;244(1):217 - 221
7 Hollstein MC, Metcalf RA, Welsh JA, Montesano R, Harris CC. Frequent mu tation of the p53 gene in human esophageal cancer. *Proc Natl Acad Sci USA*, 1990;87(12):9958 - 9961
8 Benchimol S, Pim D, Crawfod L. Radioimmunoassay of the cellular protein p53 in mouse and human cell lines.*EMBO*, 1982;7(5):1055 - 1062
9 T Soussi, Y Legros, R, Lubin K. Multifactorial analysis of p53 alteration in human cancer: a review. *Int J Cancer*, 1994;57(1):1 - 9
10 Reich NC, Levine AJ. Growth regulation of a cellular tumor antigen p53 in nontransformed cells. *Nature*, 1984;308(1):199 - 201
11 Hollstein M, Slidransky D, Yogelstein B. p53 mutation in human cancers. *Science*, 1991;253(1):49 - 53
12 Harris CC, Hollstein M. Clinical implication of the p53 tumor suppressor gene. *N Engl J Med*, 1993;329(5):1318 - 1327
13 Moll UM, Riou G, Levine AJ. Two distinct mechanisms alter p53 in breast cancer mutation and nuclear exclusion. *Proc Natl Acad Sci*, 1992;89(2):7262 - 7266