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THE STUDY ON RELATIONSHIP BETWEEN CIGARETTE SMOKING AND THE p53 PROTEIN AND P21 PROTEIN EXPRESSION IN NON-SMALL LUNG CANCER

Zhou Baosen 周宝森 He Anguang 何安光 Zhu Jijiang 朱继江
Wang Enhua 王恩华

Laboratory of Lung Cancer, Cancer Institute, China Medical University, Shenyang 110001

This paper discusses the relationship between cigarette smoking and the p53 protein and P21 protein expression by the immunohistochemical analysis in 93 cases with lung cancer in which squamous cell carcinoma accounted for 45 cases, adenocarcinoma 48 cases. The results showed that positive proportion of p53 protein expression was 74.20% (28 of 37 squamous cell carcinoma, 21 of 30 adenocarcinomas) in cigarette smoking group with lung cancers, and 38.46% (3 of 8 squamous cell carcinoma, 7 of 18 adenocarcinomas) in nonsmoking group with lung cancers. The difference was statistically significant. Odds ratio was 4.14 and confidence limits for OR was 1.42–12.52. A dose-related presents in the p53 protein expression for the smoking amount and smoking years. The positive proportion of P21 protein expression was 79.31% (21 of 28 squamous cell carcinoma, 25 of 30 adenocarcinomas) in cigarette smoking group with lung cancers, and 82.75% (10 of 11 squamous, 14 of 18 adenocarcinomas) in nonsmoking group with lung cancers, the difference was not statistically significant. But their positive proportion of P21 protein expression were very high in both groups. It was indicated that no relationship between cigarette smoking and the P21 protein expression. We suggest that the p53 gene could be a common target of tobacco-associated carcinogenesis in lung cancer.

Key word: p53 protein expression, P21 protein, Cigarette smoking, Lung cancer.

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It is well know that the occurrence of certain subtypes (squamous cell carcinoma, small cell carcinoma, large cell carcinoma) of human lung cancer is associated with cigarette smoking.[1] Tobacco is the dominant etiologic agent for at least 80 percent of lung cancer cases in China.[2] However, we do not know the mechanisms of this association and the target gene for the carcinogens in tobacco. Our purpose was to investigate the relationships between mutant p53 protein expression, P21 protein expression, and cigarette smoking in patients with non-small cell lung carcinoma.

MATERIALS AND METHODS

Patients and Tissues

Specimens of the 93 cases with non-small cell lung carcinoma was formalin-fixed paraflim-embedded of materials examined histologically, enrolled in the department of Thoracic Medicine at the China Medical University from 1992–1994. All cases were typed in accordance with the WHO classification (1981). PAS-ab staining was applicated to distinguish squamous cell carcinoma from adenocarcinoma. Information on the history of cigarette smoking, daily cigarette consumption, and the duration of cigarette smoking in patients with lung cancer were collected from the medical records.

Immunohistochemistry

Two monoclonal antibodies, D0–1 and F132 were used for immunohistochemistry. D0–1 antibodies by immunoprecipitation with a 53-kDa protein. F132 antibodies by immunoprecipitation with a 21-kDa protein. Expression of mutant p53 protein and P21 protein in non-small cell lung carcinoma (squamous cell carcinoma accounted for 45 cases, adenocarcinoma 48 cases) by IHC. Every monoclonal antibodies was utilized on the basis of pilot study of 93 tumors in which each antibody was used separately. Five micrometer section were prepared from fresh-frozen pellets of particular lung cancer specimens as previous described. Staining procedures were as follows, submerge slide in peroxidase quenching solution. Wash with PBS (5 min., 3 times). Add 100 μl of serum blocking solution to each section. Incubate for 20 min. Drain or blot off the solution. Apply 100 μl of primary antibody to each section. Incubate in moist chamber for 60 min. Rinse well with PBS (2 min., 3 times). Apply 100 μl of biotinylated second antibody to each section. Incubate for 40 min., Rinse well with PBS (2 min., 3 times). Apply 100 μl of enzyme conjugate to each section. Incubate 40 min. Rinse well with PBS (2 min., 3 times). Apply 100 μl of substrate-chromogen mixture to each section. Incubate 10 min. Rinse well with distilled water. Counterstain the slides with hematoxylin. Wash slides in tap water. Put slides into PBS until blue (approx. 30 seconds) Rinse in distilled water. Apply 100 μl of mounting solution to the slide and mount with coverslip. Control Slides: Substitute PBS for primary antibody as control. Results: Monoclonal antibodies of D0–1 and F132 immunostained slides were evaluated by light microscopy. For D0–1 antibodies, we determined a proportion of positive staining tumor cell nuclei, more than 5% positive staining tumor cell nuclei were positive, the other were negative (Figure 1, 2). For F132 antibodies, we determined a proportion of positive staining tumor cytoplasm, more than 10% positive staining tumor cytoplasm were positive, the other were negative (Figure 3, 4).

Statistical Analysis

The data were entered Fox Base and then analyzed for statistical significance by using the Epi-infor software package. For estimation of Odds Ratios (OR), Mantel-Haenszel method were fitted to the data with the Epi-infor software package. In all analyses, there was an adjustment for types of histology with lung cancer.

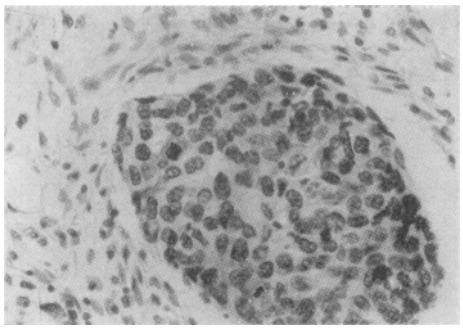

Fig. 1. p53 positive in nuclei of squamous cell carcinoma of lung, 400 ×.

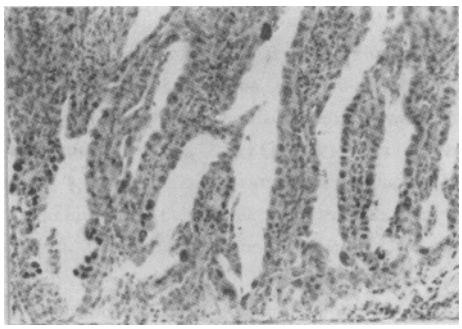

Fig. 2. p53 positive in nuclei of adenocarcinoma of lung, 200 ×.

RESULTS

The Relationship between Cigarette Smoking and the p53 Protein Expression in Patients with Non-small Cell Carcinoma

In this present study, statistical analysis of p53 protein expression was performed to correlate it with smoking history in lung cancer cases after completing immunohistochemical analysis of p53 (Table 1). Patients with squamous cell carcinoma from 28 (75.67%) of 37 smokers exhibited positive, whereas

from 3 (37.5%) of 8 nonsmokers exhibited positive staining (P=0.04). The difference was statistically significant. Results showed that cigarette smoking increased the risk for p53 expression in squamous carcinoma (OR=5.19). Patients with adenocarcinoma from 21 (70.00%) of 30 smokers exhibited positive, whereas from 7 (38.89%) of 18 nonsmokers exhibited positive staining (P=0.03). The difference was significant. Results showed that cigarette smoking increased the risk for p53 protein expression in adenocarcinoma (R=3.67). Significant dose-response patterns between cigarette smoking/day, smoke years and risk of p53 protein expression in squamous carcinoma and adenocarcinoma were observed. The excess risk of p53 protein expression for the smoking history reached 4.36-fold for squamous carcinoma and adenocarcinoma, adjust OR was 4.14-fold (1.42–12.52) by using the Mantel-Haenszel methods.

Table 1. The relationship between cigarette smoking and the p53 protein expression in non-small cell lung cancer

		p53 (+)	p53 (−)	Rate of positive (%)	OR	OR 95% CI	P
Sq.							
Nonsmokers		3	5	37.50	1.00		
Smokers		28	9	75.67	5.19	1.82–23.06	0.040
Smoke amount	≥ 20	21	2	91.30	17.50	2.92–104.74	0.002
	< 20	7	7	50.00	1.67	0.28–9.87	0.571
Smoke years	≥ 10	28	8	77.78	5.83	1.26–26.93	0.025
	< 10	0	1	---	---		
Ade.							
Nonsmokers		7	11	38.89	1.00		
Smokers		21	9	70.00	3.67	0.91–15.43	0.030
Smoke amount	≥ 20	15	4	78.94	5.89	1.45–23.92	0.014
	< 20	6	5	54.55	1.89	0.41–8.55	0.411
Smoke years	≥ 10	18	8	69.23	3.54	1.02–12.24	0.048
	< 10	3	1	75.00	4.71	0.46–47.74	0.189

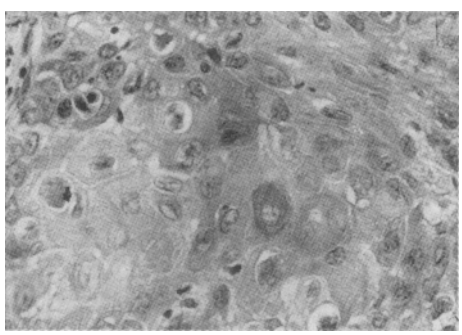

Fig. 3. P21 positive in cytoplasm of squamous cell carcinoma of lung, 400 ×.

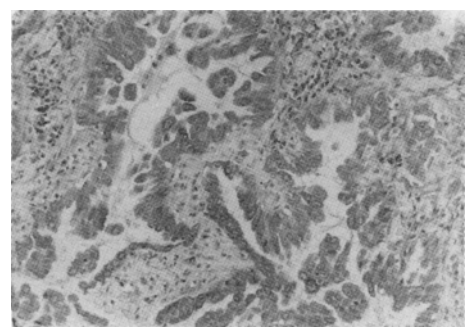

Fig. 4. P21 positive in cytoplasm of adenocarcinoma of lung, 200 ×.

The Relationship between Cigarette Smoking and the P21 Protein in Patients with Non-small Cell Lung Carcinoma

In the squamous cell carcinoma, 75% of the smokers and 90.91% of the nonsmokers were exhibited positive of P21 protein. In the adenocarcinoma, 83.33% of the smokers and 77.78% of the nonsmokers were exhibited positive of P21 protein. In both histologic types, we could not establish a statistically significant association between cigarette smoking and P21 protein (OR=0.79, P=0.94) (Table 2).

Table 2. The relationship between cigarette smoking and the P21 protein in non-small cell lung carcinoma

		p53 (+)	p53 (−)	Rate of positive (%)	OR	OR 95% CI	P
Sq.							
Nonsmokers		10	1	90.91	1.00		
Smokers		21	7	75.00	0.30	0.04–2.52	0.268
Smoke amount	≥ 20	12	8	60.00	0.15	0.02–1.16	0.069
	< 20	9	2	81.82	0.45	0.03–5.52	0.534
Smoke years	≥ 10	21	10	67.74	0.21	0.03–1.61	0.133
	< 10	0	0	---	---		
Ade.							
Nonsmokers		14	4	77.78	1.00		
Smokers		25	5	83.33	1.43	0.33–6.17	0.633
Smoke amount	≥ 20	14	5	73.68	0.80	0.17–3.75	0.772
	< 20	11	0	---	---	------	0.411
Smoke years	≥ 10	20	4	83.33	1.43	0.31–6.61	0.650
	< 10	5	1	83.33	1.43	0.12–17.05	0.772

DISCUSSION

It is well known that molecular mechanism involved in the development of human tumors are loss of function of cancer suppressor gene and mutation of oncogene. Takahashi et al. found the p53 gene was a frequent target of genetic abnormalities in lung cancer.[3] The p53 gene is a tumor-suppressor gene that encodes a 53-kDa nuclear phosphoprotein that binds to the large T antigen of the DNA tumor virus, simian virus 40 (SV40), appears to play an essential role in the negative regulation of G1-S phase transition of the cell cycle. Normal p53 negatively regulates cell growth and division, whereas mutated forms can stimulate cell division.[4] The p53 mutations may therefore function to promote growth of cancer cells. Normal p53 gene protein dose not accumulate in cell because of its short half-life. Missense mutations in the p53 gene, however, significantly increase the half-life of the p53 protein, leading to its accumulation in cancer cells. Immunohistochemical detection of this abnormal p53 protein has been shown to be a powerful means to identify p53 missense mutations that are not restricted to a single site. It is known that wild type p53 expression is not detectable in normal cells immunohistochemically. The study shows that there are obvious associations between p53 protein expression in NSCLC and a patient's smoking history. Odds ratio is 4.14 folds (1.42–12.52). This result was the same as Mitsudomi T's result[5] and Hirotoshi DA et al.'s result.[6] Mitsudomi T et al. studies showed that the p53 nucleotide mutations in lung cancers tend to differ from those in other cancers that are not as tightly linked to cigarette smoking. The former mutations are most commonly a G to T transversion (change from a purine to a pyrimidine),

whereas the latter are typically a G to A transition. Vahakangas KH et al.[7] have studied the p53 gene mutation in 19 patients with lung cancer who had been exposed to radon, another environmental agent associated with the development of lung cancer, while working underground as miners. Tumor specimen studied revealed p53 mutation in 7 of 19 patients (37%). None of those mutations were the G to T transversion commonly seen in lung cancer. Probably because the genetic lesions in these tumors were associated with a different environmental pathogen (radon) than the lesions typically found in patients who develop lung cancer after chronic cigarette smoking. We suggest that the p53 gene could be a common target of tobacco-associated carcinogenesis in lung cancer.

The ras oncogenies code for 188 or 189 amino acid proteins with a molecular weigh of 21,000. So the ras proteins is called P21 protein that normally bind guanine nucleotide (guanosine triphosphate or diphosphate), have guanosine triphosphatase activity, and play a role in the transduction of signals across cellular membranes, thereby regulating cellular proliferation.[8] Slebos RJC et al. studies showed adenocarcinomas of the lung with K-ras mutation occurred more frequently in smokers (8/27) than in nonsmokers (2/27) (P=0.044).[9] In our study, tumor specimen revealed P21 protein in 46 of 58 smokers (79%). 24 of 29 nonsmoker (82%). Both of smokers and nonsmokers are high positive proportion of P21 protein. We didn't find that the relationship between P21 protein and cigarette smoking in patients with lung cancer. Rodenhuis S et al. discovered showed that a ras oncogene is mutated in approximately 20% of NSCLC tumors and 30% of NSCLC cell lines.[10] So immunohistochemical detection of this abnormal P21 protein has been shown not to be a means to identify ras oncogene mutation. About the relationship between ras oncogene mutation and cigarette smoking in patient, we are further studying by method of PCR.

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