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EXPRESSION OF P53 AND C-MYC IN MOUSE LUNG CANCER INDUCED BY COAL BURNING

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Previous epidemiological studies have shown association between coal burning and human lung cancer. To confirm relationship between coal burning and lung cancer formation and progression the expression of p53 and c-myc in 13 mouse lung cancer induced by coal burning smoke and 5 mouse lung tissue control was studied by DNA-RNA in situ hybridization (ISH). Nine of 13 specimens showed c-myc overexpression but it occurred only 1 of adjacent tissue. There was over pression of p53 mRNA in all 13 lung cancer and 5 adjacent tissue. None in the controls was expression of p53 and c-myc detected. When compared to controls, there was significant higher expression of c-myc gene (P=0.002) and p53 gene (P=0.0001).

The results confirm that overexpression of p53 and c-myc are common molecular events of lung cancer by coal burning smoke and provide further evidence that smoke from coal burning is a causative agent of lung cancer.

Key words: Coal burning, p53 gene, *in situ* hybridization, Lung cancer, Oncogene

Alterations of p53 are one of the most common molecular changes found in all types of lung cancer at frequencies of 75% in small cell lung cancer (SCLC) and 50% in non small cell lung cancer (NSCLC)[1,2] Mutated p53 alleles typically contain missense single-base substitutions within exons 5 — 8 and encode abnormally stable p53 proteins that accumulate to high levels in tumor cell nuclei.[2]

Human lung cancer carry multiple genetic changes in tumor suppressor genes including p53 as well as dominant oncogene such as c-myc.[3,4]

Accumulating epidemiology evidence indicates that coal smoke can induce lung cancer in human. In this report, we have demonstrated association between lung cancer formation induced by coal smoke and expression of p53 and c-myc, and the biological effect of the wild-type p53 by coal smoke.

MATERIALS AND METHODS

Animal Experiment

A total of 240 Kunming mice were divided into four groups including 3 experimental groups according to the weights of burning coal (160g, 105g, 60g) and 1 control group. Animals were 13± 1g weight, 40 days, half of male and female. The mice of experimental groups were exposed to smoke of burning coal for sixty minutes per day for two years. Sixty mice of the control group were fed in daily routine without exposing to burning coal. The specimens were fixed with neutral formalin and processed in paraffin wax. Thirteen samples with obvious sign of lung cancer and adjacent tissues were taken from the experimental groups. Five normal lung

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tissue samples were obtained from the control.

In Situ Hybridization

The cDNA probe biotinylated was purchased from Chinese Army Medical Academy of Science. *In situ* hybridization (ISH) and detection system 8250 SA cot was the product of BRL Company (USA). The sections of 3 μm thickness were bound to slide which were subbed with poly-lysine in this experiment. Specifically tissue sections were digested with proteinase K.DNA-RNA hybrids were demonstrated with an alkaline phosphatase and streptavidin conjugate nitroblue terazolium (NBT) detection system. The technological process details to references.[5] Observation with microscope, positive particulate products showing blue-violet were dispersed in cell nucleus and cytoplasm. According to the number of positive cells, the expression level of p53 and c-myc gene was recorded as negative (−), positive (+), strong positive (++), very strong positive (+++), negative cells was light green because of counterstain with methylene green. Control group: 1) blank control without probe of every specimen section; 2) positive control; 3) normal lung tissue control without exposing to coal smoke; 4) adjacent tissue control of every lung cancer.

RESULTS

When biotinylated probes and NBT detection system were used on the method of ISH, the background signal due to endogenous biotin and alkaline phosphatase was present. Therefore baking sections embedded with paraffin 65 ℃ for 1.5 hours made endogenous biotin and enzyme inactive. False positivity was eliminated because of using section without probe as blank control and adjacent tissue section from same specimens as self-control.

The lung cancer induced by coal smoke were all adenocarcinomas. The incidence rate of lung cancer was 24.3% for 160g-coal group, 12.8% for 105g-coal group, 9.4% for 60g-coal group, and 3.6% for the control group respectively. There were significant difference between the incidence rate of lung cancer in experiment group and control group ($P<0.05$). There was dose-reaction relationship in experimental group and there was significance by trend test ($P>0.05$). Two cases of lung cancers in control was indicated contain natural incidence rate of lung cancer in Kunming Mouse, which was in the late stage, very small node like a point. But, the lung cancer in experiment group were in the early stage, large node like ball. The diameter of 13 elected lung cancer from experiment group was over 0.5 cm, 0.59 cm ± 0.18 cm ($\chi \pm S$).

The results of ISH are shown in Table 1 and 2. There was overexpression of p53 mRNA in 13 lung cancer samples and 5 of the adjacent tissue samples, which is shown in Table 1. Likewise, c-myc was found to be overexpressed in 9 of 13 lung cancer samples and 1 of 13 adjacent tissue samples(Table 2). By contrast no expression of p53 and c-myc in control samples was detected out. There was significant difference between c-myc. p53 expression in lung cancer and adjacent of experiment group; that in cancer tissue of experiment group and lung tissue of control group ($P<0.001$). The expression of two genes in cancer tissue was stronger than that in adjacent tissue. The positive products of c-myc gene was observed in cytoplasm. The positive products of p53 gene was observed in cytoplasm as well as nucleus.

Table 1. c-myc expression in lung cancer and control

Group	Number	c-myc expression				Positive rate (%)
		−	+	++	+++	
Experiment						
lung cancer	13	4	3	5	1	69.23
adjacent tissue	13	12	1	0	0	7.69
control	5	5	0	0	0	0.00

Table 2. p53 expression in lung cancer and control

Group	Number	p53 expression				Positive rate(%)
		-	+	++	+++	
Experiment						
lung cancer	13	0	3	3	7	100.00
adjacent tissue	13	9	4	1	0	38. 46
Control	5	5	0	0	0	0.00

DISCUSSION

p53 gene was first considered as a dominantly acting oncogene. It is now clear that the oncogenic activity of p53 depends on the mutation of its coding sequence and that the wild-type p53 gene has a clear role in inhibit transformation of cells. It was unstable, small quantity, difficult to detection. But, mutations of p53 have effect on transformation of cells and inhibition for action of wild -type p53, very stable, overexpression with proliferation of cells. Some reports suggested that loss or mutation of p53 gene was the frequent event of genetic abnormality in lung cancer.

In this study, overexpression of p53 was observed in mouse lung cancer induced by coal smoke, none in control tissue, 5 in adjacent tissue. This results suggests that carcinogen from coal smoke mutates p53 gene. There is relationship between mutation and overexpression of p53 gene and occurrence of mouse lung cancer, and former is observed in early lung cancer.

Myc gene acts with other genes, which make cells proliferate unlimitedly and promote cell division.[6] Some experiment confirmed that mitosis promoter and tumor promoter may make expression of c-myc mRNA increase 15 ~ 40 times. In this research, overexpression of c-myc mRNA occur 9 of 13 lung cancer, none of controls, 1 of 13 adjacent tissue. The results shown that carcinogen B(a) p in coal smoke may activate c-myc gene and other oncogene, their cooperation resulted in mouse lung cancer.

In addition, we have found relationship betwwen expression of mutation p53 and expression of c-myc gene, that is positivity of c-myc in cooperation with high level expression of p53. It is common with referances.[7] And it suggests that mouse lung cancer results from cooperation of oncogene and antioncogene.

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