Chinese Journal of Cancer Research 7(1):10-14, 1995.

PROMOTION OF CHEMICAL CARCINOGENESIS AND P53 EXPRESSION BY REDUCTION OF SUPEROXIDE DISMUTASE ACTIVITY IN THE LUNG OF RAT *IN VIVO**

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In the present experiment, an inhibitor of superoxide dismutase (SOD), diethyldithiocarbamate (DETC), was used to decrease SOD activity for the observation of the relation between SOD activity and carcinogenesis and the expression of P53 protein *in vivo*. 144 Wistar rats were used for the present study. The results showed that the SOD activity reduction by DETC resulted markedly in the promotion of the carcinogenesis and the expression of P53 protein in the lung tissues, but the increase of SOD activity by the addition of plus SOD inhibited the pathological changes significantly. The frequency of the pathological lesions and positive P53 expression are 36/42 and 8/42 in the animals without DETC and SOD; 16/52 and 4/52 in the animals with SOD and 46/50 and 26/50 in the animals with DETC respectively. The results reported in this paper suggest that: (1) the decrease of SOD activity enhanced the carcinogenesis induced by chemical carcinogen; (2) P53 gene may be associated with the process of tumorigenesis; and (3) at the same time the abnormal expression of P53 protein may be associated with the transition from premalignant lesions to carcinoma.

Key words: Superoxide dismutase, Carcinogenesis, Gene expression, P53, Lung, rat.

Superoxide dismutase (SOD), a scavenger for free radicals, plays an important role in the maintenance of available concentration of free radicals in cells.[1] Previous studies had shown that the radiosensity of the cells cultured could be increased by an inhibitor of SOD activity *in vitro*, but plus SOD could inhibit significantly the malignant damages induced by physical and chemical factors. Although the conjecture that the cancer development might be related to the reduction of SOD activity, which could result in the excessive accumulation of oxygen free radicals and then the occurrence of genetic damage and finally the cancer development of the cells, the hypothesis has not been confirmed by animal experiment *in vivo*.

P53 gene is thought as a tumor suppressor gene.[2,3] The mutation or abnormal expression of P53 gene are associated with cancer development and progression.[4-6] Several investigators have demonstrated that the abnormal expression of P53 protein encoded by P53 gene, which localized on the p-arm of chromosome 17, employing hybridization and immunohistochemical techniques.[7-11] The results of these analysis showed that the accumulation of P53 protein in cells may be associated with the transition form premalig-

Accepted May 15, 1994.
*The project was supported by National Natural Science Foundation of China (No. 39070383).

nant lesions to carcinoma and the malignant grades of cancers and the prognosis in the patients with cancers. Therefore some studies suggest that P53 gene may play an important role in the pathogenesis of solid tumors.

In this experiment, an inhibitor of SOD activity, diethyldithiocarbamate (DETC), was used to decrease SOD activity *in vivo* in rats to verify the exact effect of SOD in carcinogenesis and to observe the P53 expression to gain a better understanding of P53 gene in the process of tumorigenesis.

MATERIALS AND METHODS

Animals

One hundred and forty-four Wistar rats, half male and half female, weighing 150–200 g were used for the present experiment.

Carcinogen

750 mg of 3-methylcholanthrene (3-MCA) was obtained from Sigma (USA) and dissolved in 15 ml idoized oil for carcinogenesis.

Compounds

Diethyldithiocarbamate (DETC) was purchased from Beijing Chemical Corp. Bovine copper and zinc-containing superoxide dismutase (Cu Zn SOD) from Shanghai Biochemistry Institute of Academy Sinica.

Experimental Groups

One hundred and forty-four rats were divided randomly into three groups. In group control, the animals were not treated by SOD and DETC; in group SOD, the animals were given by SOD (6,000 U/each time for one rat); and in group DETC, the animals were given by DETC (20 mg/each time for one rat).

Carcinogenesis Procedure

The carcinogenesis was processed by the method of the induction of lung carcinoma by intralobar bronchial instillation of idoized oil in rats as our previously described.[12] Each rat was received 50 mg 3-MCA dissolved in 0.1 ml idoized oil.

Administration of SOD and DETC

According to the above-mentioned groups, SOD and DETC were injected by intramuscle on the 24th h before the carcinogenesis, at days 4, 7, 11, 15, 19, 23 after the carcinogenesis and the first day before the animals were killed respectively.

Macroscopic and Light Microscopic Observation for Tumor Development

At the fortyth day after the carcinogenesis, the all animals were killed by inspiration of ethol. The gross morphological and histological changes of the lung were examined to analysis the lesions induced by the carcinogen. The detail method of the examination was same as the previously description.[12]

Detection of SOD Activity

When the animals were killed the SOD activity in the peripheral blood and the tissues of heart, lung, liver, kidney and spleen was detected respectively as previously described.

Immunohistochemistry for the Expression of P53 Protein

PAb 1801 monoclonal antibody which is specific for murine and human P53 was from Oncogene Science, Inc. NY, USA. One hundred and forty-four lung tissues from the rats were fixed, sectioned, stained and analyzed by the immunohistochemical detection of P53 protein in paraffin-embedded tissues.

RESULTS

Pathological Lesions of the Lung

The results of carcinogenesis test in each group, including the premalignant lesions and carcinoma of

the bronchia are summarized in Table 1.

In group DETC, the pathological lesions were much more serious than in group control and group SOD.

The above-mentioned results were analyzed by statistics (Raddit analysis), the significant differences among the groups were confirmed (Table 1).

Detection of SOD Activity

The detected results showed that the SOD activity in the peripheral blood and the tissues was decreased markedly in group DETC, but the increased SOD activity was detected in the peripheral blood and lung tissues in group SOD (Table 2).

Table 1. Promotion of the decreased SOD activity on the carcinogenesis induced by 3-MCA in rat bronchia (Raddit analysis)

Group	Number of rats studied	Rats with negative	Rats with hyperplasia	Rats with metasplasia	Rats with dysplasia	Rats with carcinoma	*P*
Control	42 (100)	8 (19.0)	23 (54.8)	4 (9.6)	6 (14.3)	1 (2.4)	<0.01
SOD	52 (100)	36 (69.2)	11 (21.2)	4 (7.7)	1 (2.0)	0 (0)	<0.01
DETC	50 (100)	4 (8.0)	2 (52.0)	7 (14.0)	10 (20.0)	3 (6.0)	<0.01

Table 2. SOD activity of the peripheral blood and some tissues in the rats

Group	SOD activity in blood ($\bar{x} \pm s$) (U/gHb)	SOD activity in tissues ($\bar{x} \pm s$) (U/g wet weight)				
		heart	liver	spleen	kidney	lung
Control	15711± 309*	528± 43	1208± 21	1016± 86	944± 61	633± 85
SOD	17550± 287*	561± 78	1069± 26	977± 38	903± 99	829± 69*
DETC	9560± 377**	384± 21**	628± 25**	498± 23**	546± 38**	460±97**

* P <0.01 ** P <0.05

Expression of P53 Protein in Different Pathological Lesio

The positive expression of P53 protein was observed in 38 of 144 rats in the lungs. The results showed that the abnormal expression of P53 protein was associated with the transition from the premaignant lesions to carcinoma (Table 3).

Expression of P53 Protein in the Groups

The frequency of P53 expression varies in the experimental groups. The high expression frequency of P53 protein could be seen is group DETC, in which is much higher than that in group control and group SOD. The results showed that DETC, which inhibited significantly SOD activity, promoted the carcinoge-

Table 3. Expression of P53 protein in different pathological lesions induced by 3-MCA in rat lung

Type of lesions	Number of rats with lesions	P53 + (%)	P53 - (%)	*P*
Negative	48	0 (0)	48 (100)	< 0.01
Hyperplasia	60	13 (22)	47 (78)	< 0.01
Metasplasia	15	7 (47)	8 (53)	< 0.01
Dysplasia	17	14 (82)	3 (18)	< 0.01
Carcinoma	4	4 (100)	0 (0)	< 0.01

Table 4. Expression of P53 protein in the experimental groups in different lesions

Groups	Negative	Hyperplasia	Metasplasia	Dysplasia	Carcinoma	Total P53 + (%)	*P*
Control	0/8 (0)	2/23 (9)	1/4 (25)	4/6 (67)	1/1 (100)	8/42 (19)	< 0.01
SOD	0/36 (0)	2/11 (18)	2/4 (50)	0/1 (0)	0/0 (0)	4/52 (8)	< 0.01
DETC	0/4 (0)	9/26 (35)	4/7 (57)	10/10 (100)	3/3 (100)	26/50 (52)	< 0.01

nesis induced by chemical carcinogen and at the same time showed that P53 gene plays an important role in the pathogenesis of tumors (Table 4).

DISCUSSION

In the present experiment, it was observed that the pathological lesions of the lung tissues induced by the carcinogen could be promoted by the decrease of SOD activity, but inhibited by the increase of SOD activity.

Considered generally, free radicals may play an important role in carcinogenesis and SOD, which is a scavenger for oxygen free radicals, might be associated with the inhibition of tumor development due to the elimanation of oxygen free radicals. In our previous studies, we have demonstrated the importance of SOD activity in cancer metastasis, cell differentiation and chemotherapy.[13] In this experiment, we first observed the relation between SOD activity and chemical carcinogenesis *in vivo* and confirmed the important role of SOD activity in cancer development.

Since Lane and Crawford first discovered the P53 gene product in 1979, numberous investigations have been done on the meanings of P53 in tumors.[2, 3] Several studies suggest that the P53 gene as a tumor suppressor gene might regulate and constrain cell growth and division.[2, 3] Therefore, the alterations of human P53 gene have been confirmed and considered as one of the most frequent types of genetic alterations in many types of human cancers.[4–6] At the same time, the mutation of the P53 gene was commonly accompanied by the accumulation of the P53 protein.[7–12, 14] Since the establishment of immunohistochemical method for the detection of P53 protein, the expression status of P53 protein in tumor tissues has been studied for the evaluation of the malignant grades and the respect of the P53 gene mutation.[2, 3, 14]

In this experiment, the expression of P53 protein has been observed in the premalignant lesions, including the hyperplasia, metasplasia and dysplasia of the bronchial epithelium, and squamous carcinoma (Table

3), and at the same time, the high expression frequency of P53 protein to be associated with the carcinogenesis has been observed too (Table 4). Pavellic et al. observed also the P53 expression in areas with severe dysplasia and carcinoma *in situ*.

Van Den Berg et al. reported that the expression of P53 gene may be related to the progression from the benign to malignant state in colon tumors. Other researchers have shown the increased expression of P53 in human and in animal tumors,[7,9,10] and suggested that the expression of P53 may be associated with the tumor grades and the prognosis of the patients with tumors. But some investigators do not observe the results.[10]

From the above-mentioned results, the present study suggests the available SOD activity may be advantageous for cancer prevention, and at the same time, abnormal expression of P53 protein would be observed in different stages of carcinogenesis and the expression may be associated significantly with the transition from premalignant lesions to carcinoma, and the results suggest that P53 gene may play an important role in the pathogenesis of tumors.

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