A Study of Inhibitory Mechanism of Cisplatin on Human Lung Adenocarcinoma SLC-89 Cell

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Abstract **Objective** To investigate the effect of cisplatin on proliferation, cell cycle and expression of P21 and c-myc protein of human lung adenocarcinoma SLC-89 cells, in order to find out its anticancer mechanism. **Methods** By the techniques of cell culture in vitro, SLC-89 cells were treated by Cisplatin in different concentration for 72 h, then proliferation of cell was measured by MTT method, and cell cycle and expression of P21 and c-myc protein were observed by Flow-Cytometry (FCM) respectively. **Results** Cisplatin could obviously inhibit proliferation of SLC-89 cell, and IC_{50} value for Cisplatin treatment at 72h was 18.47μg/ml. Cisplatin could decrease S phase cells, increase G0/G1 phase cells and induce the expression of P21 and c-myc protein of SLC-89 cell with concentration–dependent pattern. **Conclusion** Cisplatin could obviously inhibit proliferation, change cell cycle distribution and induce the expression of P21 and c-myc protein of SLC-89 cell, which may be one of important mechanisms of Cisplatin´s anticancer funtion.

Key Words Cisplatin; Cell cycle; Gene expression; SLC-89

Xuanwei, Yunnan province, is one of the places with high morbidity of lung cancer in China. Since February 1989, we have cultured the human lung adenocarcinoma SLC-89 cells line which provided material for research and treatment on lung cancer in the area. Cisplatin is a chemical drug used to treat carcinoma including the lung cancer, and we investigated its effect on proliferation, cell cycle and expression of P21 and c-myc protein of human lung adenocarcinoma SLC-89 cells in order to find out the mechanism of its anticancer function.

MATERIALS AND METHODS

Meterials

SLC-89 cells were founded in February, 1989, and preserved in the Department of Medical Experiment of Kunming General Hospital of Chengdu Military Command. Cisplatin was purchased from Haikou Pharmaceutical factory. RPMI-1640 was obtained from GIBCO company. P21 and c-myc monoclone antibody were provided by Dako company. Flow-cytometry was the COULTER EPICS×L (US), and was DYNEX REVELATION3.2(US).

Experimental methods

SLC-89 cells were cultured according to the method described by Chen Zhilong, et al [1]. IC_{50} value for Cisplatin treatment at 72 h was measured by MTT assay according to the method described by Pang Rongqing, et al [2]. A total of 4.5×10^4/ml SLC-89 cell suspension were transferred into 50 ml culture flask and incubated for 24 h, and then Cisplatin in different concentration were added to the control group and the experimental group respectively. SLC-89 cells were incubated with Cisplatin in different concentration for 72 h and then were collected with trypsin-EDTA to study the mechanisms of Cisplatin´s anticancer funtion.

Measurement of expression rate of P21 and c-myc gene protein.

SLC-89 cells collected were rinsed by phosphate buffered saline(PBS) twice, the cells were suspended by PBS. P21 and c-myc monoclone antibody were added to both groups respectively. After incubation for 30 min at 37℃, 100 μl goat-anti-rat FITC-IgG were added and incubated for 30 min at 37℃, and centrifuged at 2000rpm for 5 min and then the supernatant was removed. Centrifuguation was repeated once, and the cells were resuspended in 100 μl PBS and the expression rate of P21 and c-myc gene protein were measured after the suspension. The cell marked with fluorescence was regarded as positive cells. The proportion of the

positive cells was the expression rate of P21 and c–myc gene protein.

Analysis of cell cycle

The cells collected were rinsed by PBS twice and immobilized with 70% cold alcohol and centrifuged for 5 min at 300×g. The cells resuspended in a 0.1% Triton X-100/PBS solution to strip the nuclei and the concentration was adjusted to 10^5–10^7/ml. The cell suspension was filtered and 0.5% Rnase was added. The nuclear DNA was stained with propidium iodide 50 μg/ml. A flow cytometer was used to determine the amount of nuclear DNA for 10000 cells in each specimen, and the proportion of cells in each cycle phase was calculated using the software program.

Statistical methods

The data was showed $\bar{x}\pm s$. Comparison between groups was performed using t test, and all calculations were performed by SPSS 8.0.

RESULTS

Susceptibility of SLC–89 to Cisplatin

Cisplatin could effectively inhibit proliferation of SLC-89 cells and change the appearance of the cells or separate the cells in the culture medium. IC_{50} value for Cisplatin treatment at 72 h was 18.47 μg/ml, which showed that SLC-89 cells were susceptible to Cisplatin.

Effect of Cisplatin on expression of P21 and c-myc gene protein.

While inhibition of SLC-89 cell, Cisplatin also could induce expression of the cell P21 and c-myc gene protein with concentrition-dependent pattern, which was obvious difference compared to the control group (table 1).

Effect of Cisplatin on cell cycle

With the rising of Cisplatin concentration, the proportion of G_0/G_1 phase cells increased and S phase cells decreased markedly, which demonstrated Cisplatin not only could inhibit replication of DNA by arresting the cells to G_0/G_1 phase, but also there was dependent relation between the inhibition and the concentration of Cisplatin.

DISCUSSION

At present, lung cancer has become one of the major reason for death from carcinoma worldwide, and the chemotherapy based on Cisplatin has been one of the major treatment methods. Cisplatin, an inorganic metallic coordination compound whose molecular weight is 300.05×10^3, plays an important anticancer role in blocking replication of DNA by binding DNA of carcinoma cell. The cytological basis of prevention and treatment of carcinoma is the inhibition of proliferation and differentiation of carcinoma cell. Peoples have known that proliferation and differentiation of cell were modified by many factors such as c-myc and P21 and other genes which play important role in modulating and controlling proliferation and differentiation of carcinoma cell.

C-myc is the early gene in cell cycle which codes a Myc in nucleus. Researches[3,4] show that myc could combined with DNA and take part in the replication of DNA. Myc is also involved in the formation of transcription fators´ network. It plays an important role in accelerating cell proliferation and controlling cell differentiation and apop-

Table 1 Effect of Cisplatin on cell cycle and expression of P21 and c–myc protein of human lung adenocarcinoma SLC–89 cell ($\bar{x}\pm s$)

Groups	concentration (μg/ml)	Proportion of the positive cells(%)		Cell cyle(%)		
		P21	c–myc	G0/G1	S	G2/M
Control	0	28.01±0.98	31.02±1.92	61.98±2.09	14.98±1.88	23.04±2.25
Cisplatin	5	32.13±3.01*	33.61±4.38Δ	66.56±5.31*	11.49±5.99*	21.95±4.36Δ
	10	40.01±4.71*	38.19±6.20*	70.01±5.82*	9.01±4.96*	20.98±5.67*
	15	43.94±6.02*	45.09±5.49*	75.30±6.19*	6.01±5.49*	18.69±6.08*

Note: Cisplatin groups compared with the control group (*$P<0.01$; Δ$P<0.05$)

tosis. The function region that promote proliferation and induce apoptosis of c-myc is the same. When expression reinforce, its function depend on the accepted foreign signals. The expression of c-myc is regulated down as many types of cell are induced differentiation. Our results show that the c-myc expression of SLC-89 is regulated up after the treatment by Cisplatin in different concentration for 72 h.The reason may be due to the varies expressions of c-myc in different cell cycle.

P21 is coded by the ras gene family. It is constituted by 188 amino acid and its molecular weight is 20.8 kDa. P21 is synthesized in cytoplasm. After translation, it transfer to plasma membrane and located at inner surface of cell membrane. P21 has some relations with the convection of foreign signals and the regulation of second messenger system. It belongs to the transducin of membrane signal. Some researches [5,6] showed that active ras induce differentiation by accelerating the activity of GATA-1. Moreover, its product P21 could inhibit cell proliferation. Our results illustrated P21 expression of SLC-89 regulated up after 72 h treatment by Cisplatin. It clarifies that Cisplain inhibit SLC-89 by inducing the expression of P21 which could inhibit cell proliferation.

Our results showed that Cisplatin could obviously inhibit proliferation of SLC-89 cell, and change distribution of cell cycle and decrease S phase cells with concentration-dependent fashion. This result was correspondence to the majority of reported results, but was opposite to the result reported by Ji Xuemei et al [7]. So, the results indicated that the tendency to Cisplatin modulating cell cycle of carcinoma cell was conformable yet, and the reasons could conclude: ① The mechanism of canceration of carcinomas may be different because the different cell lines were studied in different reports; ② the cell cycle was modulated by many factors, for example, telomerase, however, there are difference between the tendency of effect of chemotherapeutic agent on telomerase of carcinoma cell[8]. It make us happy that researches on different tumor cells have made significant progress in recent years. The results of this study on human lung adenocarcinoma SLC-89 cell in Xuanwei showed that Cisplatin could inhibit lung cancer by inducing the expression of c-myc and P21, Which may give some directions to the clinical practices of prevention and cure of lung cancer in Xuanwei.

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