Effect of NFkB Family Proteins on Growth and Drug–Resistance of Human Ovarian Cancer Cell Lines

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Abstract **Objective** To investigate the relationship between the expressions of NFkB Family proteins in ovarian cancer cell lines with chemotherapy resistance of ovarian cell lines. **Methods** 13 ovarian cancer cell lines were cultured, and then protein was extracted separately from cytoplasm and nucleus. Using Western blotting and MTT chemosensitive testing, the relationship was observed between the expressions of NFkB family proteins in ovarian cancer cell lines with chemotherapy resistance of ovarian cell. **Results** It was found that the expression rate of P65, P50 and IkB in cytoplasm was 76.9%, 81.8% and 84.6% respectively, and the positive expression of P65 and P50 was also found in the nucleus, with the positive rate being 15.3%, 45.5% respectively. In OV–MZ–5 and OV–MZ–2774, the expression of P65 was positive and their IC50 reached a significant value. **Conclusions** It was concluded that P50 and P65 affected the growth and development of ovarian cancer cells with the effect of P50 being more obvious. P65 had a close relationship with the chemotherapy resistance of ovarian cancer cells, and thus P65 could be expected to be a new marker in the observation of prognosis.

Key Words NFkB family protein; ovarian cancer cells; protein extract, western blotting; MTT chemosensitive testing

The malignant ovarian tumor has a high incidence and mortality rate in gynecology tumor. The main treatment is to resect tumor and chemotherapy after operation. But there is a lower rate of survival and cure. The drug resistance is thought to be the major cause for failure of treatment. It showed there was a correlation between the drug resistance and lower sensitive to apoptosis[1]. Nuclear factor kappa B (NFkB) is an important transcription factor and NFkB maybe has a relationship with proliferation of tumor cell and suppression of apoptosis [2]. The aim of this paper was to study the relationship between the NFkB family proteins with the chemotherapy resistance of ovarian cell.

MATERIALS AND METHODS

Cell lines and culture conditions

Thirteen human ovarian cell lines were derived from ovarian cancer tissue. The cells were cultured in DMEM media (containing 10% fetal bovine serum, 2 mM l–glutamine, 50 U/ml Penicillin and 50 μg/ml Streptomycin) at 37℃ in 5% CO_2 incubator.

Protein extraction

2×10^6 cells were harvested in PBS and washed twice. The protein was extracted by the nuclear and cytoplasm extraction reagent (RIERCE company, 78833). The protein was quantified by BCA protein Kit (PIERCE company, 23225).

Western blot

The protein extraction was added into 10% SDS–PAGE and electrotransferred to ECL–Nitrocellulose membranes (Amersham Pharmacia Biotech.) then was blocked for 5% of non–fat milk. After incubation with primary antibodies and the secondary antibodies, immunoreactive proteins were detected by ECL reagent according to the manufacturer´s instruction. The primary antibodies: P65(A: SC–109); P50(H–119): SC–7178; IkB–α(C–21): SC–371 (purchased from Santa Cruz Biotech, CA)

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MTT

Cells were grown in 96-well plates 1×10^4 cells/well. Adriamycin (ADM) concentrations were adjusted into 20μg/ml, 15μg/ml, 10ug/me, 7.5μg/ml, 5μg/ml and 1μg/ml respectively with PBS. 10μg/ml of different dilutions of drugs was added to each well after incubation at 37℃ for 28h in a humidified 5% CO_2 air. MTT (5mg/ml) 10μl was added into each well, incubated at 37℃ for 4 h in a humidified 5% CO_2 air, the supernatant was discarded, and DMSO was added to each well. Absorbance of the well was read in a scanning well microculture plate reader (570nm). IC50 was calculated as the concentration of drug that 50% cells were inhibited comparing with untreated control cells.

RESULTS

The expression of NFkB and IkB-α in ovarian cancer cell line

The expression of P65, P50 and IkB-α was in both of cytoplasm and nuclear, but IKB-α located mainly in cytoplasm. It was found positive expression of P65, P50 in nuclear and the expression of P50 were remarkable (Table 1).

MTT in ovarian cancer cell lines

MTT experiment was made in 10 ovarian cancer cell lines. From table 2, it was found that cell line OV-MZ-5 and OV-MZ-2774 had a high IC50 value and the expression of P65 was positive. But in those 5 cell lines whose expression of P50 was positive in nuclear, the IC50 value has no regularity.

The relationship of IC50 level and NFkB

IC50 level was higher in cell lines that expressed P65 positive in nucleus than those P65 negative in nucleus. But the IC50 level was similar in those cell lines that P50 expressed positive or negative in nucleus (Table 3).

Table 2 The IC50 value of different ovarian cell lines

Cell Lines	IC50 (μg/ml)
OV-MZ-2a	10.5
OV-MZ-2b	11.5
OV-MZ-5	20
OV-MZ-6	7.5
OV-MZ-12b	4
OV-MZ-32	4
OV-MZ-33	12.5
OV-MZ-35	8.5
OV-MZ-38	8.5
OV-MZ-2774	15

DISCUSSION

NFkB is a transcription factor and its protein family includes P50/P65, P52/P100, RelA, RelB and c-rel. Most of them can form DNA-binding homodimers or heterodimers. The most common dimer, which is new specifically referred to as

Table 1 The expression of NFkB and IkB-α in ovarian cancer cell line

	Cell lines	Cytoplasm		Nuclear	
		Positive Cell lines	Positive Rate	Positive Cell lines	Positive Rate
P65	13	10	76.9%	2	15.3%
P50	11	9	81.8%	5	45.5%
IKB	13	11	84.6%	0	0

Table 3 The relationship of IC50 level and NFkB

	Positive in nucleus		Negative in nucleus	
	Cell lines	IC50 (μg/ml)	Cell lines	IC50 (μg/ml)
P65	2	17.5	8	8.5
P50	5	9.7	5	10.6

NFkB, is composed of P50 and P65 subunits.[3] In resting cells, NFkB activity is tightly controlled by a group of inhibitory proteins belonging to the IkB family called IKBs. The activation of NFkB is stimulated by some agents, resulting in degradation of IkB and release of an active NFkB, which then translocates into the nucleus where it binds to a specific DNA sites and regulates the transcriptional activity of its target genes. So the expression of NFkB protein in nucleus is defined as the activation of NFkB[4]. Active NFkB may inhibit the transcriptional of P53 and improve transcriptional of antiapoptosis genes so as to result in the proliferation of tumor cell[5]. We detected the expression of P65, IkB-α in 13 ovarian cell lines and p50 in 11 cell lines. The results showed the IkB-α located mainly in the cytoplasm. P65 and P50 expressed in both of cytoplasm and nucleus. P50 expressed high to 45.5% but P65 only 15.3% in nucleus. It suggested that both of P50 and P65 may play a role and P50 may be more remarkable in growth and development of ovarian cancer.

Two ovarian cancer cells were resistant to ADM. IC50 value are more than 15μg/ml in these 2 cell lines and all of them expressed p65 positive. We also observed that IC50 level was higher in cell lines that expressed P65 positive in nucleus than those P65 negative in nucleus. But no difference was found in the expression of P50. It showed P65 might play an important role in forming of drug resistance. The activation of P65 had a relationship with improving cell proliferation and protecting cancer cell against apoptosis. NFkB takes three general models to regulate apoptosis: to inhibit or promote apoptosis by the direct regulation of genes; to desensitizes or sensitizes a cell to apoptotic signals by the regulation of the cell cycle; to affect the life balance of the cell by interaction with cellular proteins levels[6]. That NFkB could participate in resistance to anti-cancer drugs, which may be the result of anti-apoptosis. Some experiments showed that inhibiting action of NFkB can increase the sensitive to anti-cancer drug. So it gives a new way to reduce the drug resistance by the inhibitor of NFkB[6,7].

Generally, we conclude that: both P65 and P50 have a relationship with the genesis and development fo ovarian cancer with the effect of P50 being more obvious; expression of P65 in nucleus may predict chemotherapy resistance in ovarian cancer and could be expected to be a new marker in the observation of prongnoses.

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