Effect of Tumor Necrosis Factor (TNF) On Survivin Expression In NCI-H520 Cell Strain

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Abstract: To investigate inhibitory effect of tumor necrosis factor (TNF) on survivin expression in NCI-H520 cell strain. **Methods**: The NCI-H520 cell strains were treated with TNF at different concentrations. Assayed inhibitory ratio by **MTT**), mRNA expression by semiquantitative RT-PCR, and expression of survivin by Western-blotting. **Results:** TNF obviously inhibited the growth of NCI-H520 cell strain, depending on drug concentration. Survivin expression was degraded after treated with TNF for 4 hours, while the expression of survivin mRNA had no obviously changes in NCI-H520 cell strain. **Conclusion**: TNF can inhibit the growth of NCI-H520 cell strain, which is concentration-dependent and time-dependent. Besides, TNF can decline the expression of Survivin protein, while the expressions of survivin mRNA have no obviously variation.

Keywords: Tumor necrosis factor (TNF); NCI-H520 cell strain; Survivin

Introduction

The most common malignant tumours in the world which is harmful to human health is lung cancer and its incidence increases every year. Nowadays, the incidence and mortality rate of lung cancer has the first rank among various tumors [1].Nonsmall-cell lung cancer (NCLC) accounts for majority of lung cancer cases (mainly including squamous cell carcinoma, adenocarcinoma, and large cell carcinoma). It is recently reported that the expression of Survivin in non-small cell lung cancer can induce drug tolerance, and then affect the chemotherapy. By treating NCI-H520 with TNF, this study is to investigate the effect of TNF on biological behaviour of NCI-H520 and explore the mechanism of its inhibitory effect on lung squamous cell carcinoma.

Material and Methods

Human lung cancer cell line : Cell strain of human lung squamous cell carcinoma, NCI-H520, is provided by Cell Center, Institute of Basic Medical Science, Chinese Academy of Medical Sciences.

Drug and reagent: Rat's monoclonal antibody of Survivin from Santa Cruz Company; Rat's monoclonal antibody of β-actin from Sigma Company; Peroxidase-labelled goat-anti-rabbit secondary antibody, peroxidase-labelled goat-anti-rat secondary antibody from Zhongshan Golden Bridge Biological Company in Beijing; BCA protein assay kit, Western Blot ECL development kit from Boster Company in Wuhan; Medical photographic film from Lucky Company in China; TRIzol assay and RT-PCR assay from Invitrogen company; TNF from Celstar Biological Pharmacy Company in Shanghai.

Cell culture: After resuscitation, NCI-H520 cells were inoculated into culture flask. 5mL 10% culture solution RPMI1640 containing fetal bovine serum and double antibody were added. With cap slightly unscrewed, the flask was placed in cell incubator under 37□, 5% CO_2 and saturated humidity. When the cells were in

logarithmic growth phase, they were digested and counted. The concentration was adjusted into 5×10^4/mL, and then these cells were inoculated into 96-well culture plate.

Determination of inhibitory ratio of NCI-H520 cell by MTT : All cells were inoculated into 96-well culture plate with 100μL per well. The plate was placed in CO_2 incubator for incubation. TNF at different concentration (5, 0.5, 0.05g/mL) was added into the plate after 24hrs, with four repeated wells for one concentration. Blank group and control group were also set up. 30μL MTT reagent were added into each well and mixed properly in 4, 8, 12, 24 hrs.. After 4hrs in CO_2 incubator, supernatant was discarded. 100μL dimethyl sulphoxide were added into each well. 10 min later, the plate was determined by enzyme mark instrument under 492nm emission wavelength. The inhibitory ratio was calculated according to following formula: Inhibitory ratio= 1□ (treatment group OD□blank group OD / control group OD □blank group OD)

Reverse transcription- polymerase chain reaction (RT-PCR): Total RNA of TNF at different concentration and at different action time was extracted by Trizol one-step method. 1μg total RNA was reversely transcribed by RT-PCR assay. Survivin was amplified by PCR, with β-actin amplification as internal parameter. The primer was synthesized by Da Lin-bao Biotechnological Company. The length of amplification was 188bp, and the primer sequence of surbivin was as following:

F:5'-CCCTGCCTGGCAGCCCTTTC-3'; R:5'-CTGGCTCCCAGCCTTC CA-3'

While the length of β-actin amplification was 512bp, and the primer sequence was as following:

F:5'-GGGAAATCGTGCGTGACATT-3';

R:5'-CGGACTCGTCATACTCCTGCTTG-3'

The product was tested by 2% agarose gel under viltalight lamp.

*Expression of Survivin protein by Western-blotting:*Cells at different action time were collected and splitted. Concentrations of total protein in each group were determined by BCA protein assay kit. 80μg total protein were separated by 15%SDS-PAGE gel electrophoresis, and then transferred into PVDF membrane. The membrane was sealed with TBS buffer solution containing 2% dried skim milk for 1h under room temperature. After washing, rat's monoclonal antibody of Survivin was added into the membrane. The membrane was incubated overnight under 4□. It was washed again, added with peroxidase-labelled goat-anti-rat secondary antibody, and incubated for 1h under room temperature. Then it was developed by ECL development reagent after washing, and exposed in darkroom. Treated with Stripping buffer solution, it was sealed again, and was hybridized with β-actin monoclone antibody in the same way. All straps in every group were scanned by grayscale scanner for expression of protein. The results in both groups were divided to get relative expression amount. Besides, with the relative amount of control group as 1, ratio in each group was calculated by dividing the relative amount of control group.

Results

Inhibitory effect of TNF on NCI-H520 cell: Determined cells in each group 4, 8, 12, 24hrs later respectively with MTT after treating with TNF. Poisonous assay showed

that TNF can obviously inhibit the growth of NCI-H520 cell. Moreover, with the higher concentration and longer action time, the effect was more remarkable.

Effect of TNF on expression of Survivin mRNA in NCI-H520 cell strain : Extracted total RNA based on protocol of TRIzol assay kit at 4, 8, 12, 24hrs later after treating NCI-H520 cell strain with TNF at different concentration. Reversely transcribed mRNA into cDNA, and amplified all cDNA with Survivin and β-actin primer. Tested the product by 2% agarose gel electrophoresis. The result showed no significant change in expression of Survivin mRNA.

Effect of TNF on expression of Survivin protein in NCI-H520 cell strain: Determined expression of Survivin and β-actin protein with Western-blotting at 4, 8, 12, 24hrs later after treating NCI-H520 cell strain with TNF. All straps in every group were scanned by grayscale scanner for expression of protein. The results in both groups were divided to get relative expression amount. Besides, with the relative amount of control group as 1, ratio in each group was calculated by dividing the relative amount of control group. It showed that the expression of Survivin protein began to decline from the 4h, and descended more greatly from 8h to 24h; while the expression of Survivin protein had slight variation.

Discussion

Nowadays, the incidence and mortality rate of lung cancer is ranked first among various tumors[1]. Due to several factors, most patients are at (a) advanced stage before they are diagnosed, and miss the best operative opportunity. Although chemotherapy is regarded as main treatment for lung cancer, present chemotherapeutic drugs are still expensive, with severe side effect and (lead to) drug-resistance. Restricted by various factors such as economical condition, physical status, declining therapeutic effect and so on, some patients can't complete regular course of treatment, and some can't achieve satisfactory outcome. These factors become determinant in affecting healing rate and life quality. Gradually, people(are) pay(ing) more attention on biological anti-tumor drugs, in which TNF is the most popular. TNF was discovered and named by Caswell in 1975. It has direct cytotoxic effect on various tumor cells. The most noticeable character of anti-tumor is specifically killing tumor cells in vivo and in vitro, without toxic action on normal cells[2]. The experiment in vitro confirmed that TNF-α has specific anti-tumour character, selectively killing tumor cells, which is different from cytotoxic anti-cancer drugs. Only when the concentration becomes 100-10 000 times higher than normal, can TNF-α inhibit normal cells. This highly selective feature is unequaled with most traditional antitumor drugs[3-4]. As the most effective antitumor cytokine, it is found that the main mechanism of TNF-α is leading to apoptosis of tumor cells, injury and ischemic necrosis of tumor tissues and vessels, inducing and boosting cytotoxic effect of NK cells and macrophage, mediating immunomodulation and sensitization in radiotherapy and chemotherapy. As a new member of inhibitor of apoptosis protein family (IAP family), Survivin is closely related to cell function such as division, proliferation and so on. It is highly expressed in embryonic tissue and most tumor tissue, while the expression can't be found in normal terminal differentiation tissue or tumor-adjacent tissue [5-6]. This is suggestive of relationship

between Survivin and tumor. Nearly all non-small lung cancer patients at early stage have over expression of Survivin. So it is assumed that over expression of Survivin is a valuable referential index for early diagnosis of lung cancer [7]. The expression of Survivin doesnot depend on with patients' age, gender, and pathohistology. It is dependent on stage, with higher positive rate at later stage[8]. Thus it is indicatory in judging prognosis of lung cancer [9]. It is also reported that blocking Survivin gene can mediate cell apoptosis, and targets specificity. This paves the way for a new biological anti-tumour drug. This study selected human lung squamous cell carcinoma, NCI-H520 cell strain, and treated with TNF to observe the inhibitory effect of TNF on cell growth, and the expression of both protein and mRNA of Survivin. The result indicated that TNF can obviously inhibit NCI-H520 cell strain, and the action intensity is directly proportioned to concentration and action time of drug. The effect on transcription level of Survivin mRNA was insignificant. It suggested that TNF inhibit the expression of Survivin by specifically influencing on regulation after transcription or during translation process, not from the beginning. Some genes or products of expression in tumor tissue may play a key role in the expression of Survivin. Finding out the regularly mechanism of Survivin expression can enhance the chemotherapeutic effect on lung cancer. This study has proved that TNF can obviously inhibit expression of survivin, and could strengthen chemotherapeutical effect in some drug-resistant cases. Combined therapy can provide a new path for elevating therapeutic effect of traditional chemotherapeutical drugs. Since the drug-resistant mechanism of anti-lung cancer drug is too complicated, it still need further research to figure out specific cases and treatment which can induce synergistic effect. In a word, TNF can inhibit the expression of Survivin in lung carcinoma cells to induce cellar apoptosis. This inhibition is caused by affecting the regulation of Survivin gene after transcription or translation process, not from the beginning. As a member of inhibitor of apoptosis protein family, Survivin is closely related with drug-resistance of lung carcinoma cells. It is suggested that declining expression of Survivin by TNF could suppress drug-resistance, increase the sensitivity of lung cancer into chemotherapeutic drugs, and boost the chemotherapeutic effect.

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