Affection of Neoadjuvant Chemotherapy on Cell Apoptosis and Proliferation in Breast Cancer

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Abstract **Objective** To investigate whether neoadjuvant chemotherapy could induce tumor cell apoptosis in breast cancer, and its effect on cell proliferation. **Methods** Apoptosis index (AI) is assayed by TdT-mediated dUTP nick end labeling (TUNEL) and proliferating cell nuclear antigen (PCNA) is examined by immunohistochemical labeled streptavidin biotin (LSAB) in 100 beast cancer samples. **Results** Tumor cell AI in neoadjuvant chemotherapy group ($\bar{x}$=7.47%) was significantly higher than that in control group ($\bar{x}$=4.83%), $P<0.01$. PCNA expression rate in neoadjuvant chemotherapy group ($\bar{x}$=33.71%) was significantly lower than that in control group ($\bar{x}$=51.52%), $P<0.01$. There was significant negative association between AI and PCNA in both neoadjuvant chemotherapy group and control group ($P<0.05$). **Conclusion** Neoadjuvant chemotherapy could induce tumor cell apoptosis and inhibit tumor cell proliferation in human breast cancer.

Key words Breast neoplasm; Neoadjuvant chemotherapy; Cell apoptosis; PCNA

Breast cancer is one of the most popular tumors of women. It is not only a local disease, but one disease affects the whole body. It has been proved that some chemotherapy drugs can induce tumor cell apoptosis in breast cancer in lab[1,2]. We did relevant study in human body as follows, to stuty the affection of neoadjuvant chemotherapy on cell apoptosis and proliferation in breast cacer.

MATERIAL AND METHODS

Clinical data

512 patients with breast cancer had been treated in our hospital during July 1997 to July 2000. Of them, 100 female patients who had radical operation were chosen into our study, 50 cases of neoadjuvant chemotherapy group and 50 cases of control group. All patients had been pathologically diagnosed by needle biopsy before treated. the median age of patients is 46 years old, ranging from 25 to 69 years. 46 patients were after menopause, 54 were not. Diameter of tumors are between 2 ~ 5cm in 38 cases, >5cm in 62 cases. The status of clinical staging: 20 cases of ⅡA, 18 cases of ⅡB, 46 cases of ⅢA and 16 cases of ⅢB. All patients had routine tests before operation, no one had serious disease in important organs, and no one had tumor metastases. Patients of the control group had been 1 : 1 matched-pair with the neoadjuvant chemotherapy group according to their age, clinical staging, status of menses and ER (estrogen receptor). The conditions of clinical staging, menopause or not, ER positive or not were same, and the age difference were no more than 5 years between each pair.

In neoadjuvant chemotherapy group, patients before menopause had 2 cycles of chemotherapy with CMF (CTX 500mg/m^2, MTX 30mg/m^2, 5-FU 500mg/m^2), patients after menopause had 2 cycles of CAF (CTX 500mg/m2, ADM 30mg/m^2, 5-FU 500mg/m^2) chemotherapy. They had radical operations at 2 ~ 3 weeks after the chemotherapy. Otherwise in control group, patients had not any anti-tumor therapy before their radical operations.

All breast cancer specimens were fixed in 10% formalin solution after operation, and stored by paraffin-embedded.

Experiment methods

3 pieces of 4 μm slice were cut from every paraffin-embedded specimen. One section had HE staining, the other two had immunohistochemical staining. Each staining method had positive and negative control. Positive cells had brown depositions in the nucleus, no deposition appear in negative control section (Fig1 ~ 4).

Cell apoptosis was tested by TdT-mediated dUTP nick end labeling (TUNEL), the detailed procedures are: paraffin-embedded tissue were deparaffinized with dimethylbenzene and rehy-

drated with grads of ethanol, then rehabilitate the antigen with microwave and treated with ready-to-use TUNEL solution at 37℃ for 60 minutes. Rinsed well with PBS then applied second antibody anti-digoxin complex, incubated in moist chamber at 37℃ for 30 minutes. Coloration with DAB and rinsed well with flowing water, then counter stained with hematoxylin solution, dehydrated and enveloped the slices at last. The apoptosis-test kit is produced by Bochringer Mannheim Co. German. Apoptotic index (AI) = number of apoptosis cells/number of all cells calculated (× 100%).

Proliferating cell nuclear antigen (PCNA) was tested by labeled streptavidin biotin (LSAB), the PCNA-test kit is produced by DAKO Co. America. The detailed procedures were done according to explaining of the kit. PCNA positive rate = number of PCNA positive cells/number of all cells calculated (× 100%).

Statistical methods

All data were input into computer and made statistical analysis with SSPS 8.0. AI (%) and PCNA positive rate(%) of breast cancer was described with mean and standard deviation($\bar{x} \pm s$). We compared AI and PCNA expression between neoadjuvant chemotherapy group and control group using paired sample t-test. Besides, we did linear correlation analysis between AI and PCNA of the two groups.

RESULTS

AI of breast cancer

In neoadjuvant chemotherapy group, the highest AI was 9.34%, and the lowest was 2.15%, $\bar{x}$=(7.47 ± 2.86)%. In control group, the highest AI was 6.24%, the lowest was 1.95%, $\bar{x}$=(4.83 ± 2.11)%. The difference of AI between the two group had obviously statistical significance (P<0.01).

PCNA expression in breast cancer

In neoadjuvant chemotherapy group, the highest PCNA positive rate was 68.2%, and the lowest was 10.1%, $\bar{x}$=(33.71 ± 6.81)%. In control group, the highest PCNA positive rate was 81.4%, the lowest was 20.8%, $\bar{x}$=(51.52 ± 10.23)%. PCNA positive rate of breast cancer had statistical difference between the two groups. P<0.01.

Relation between AI and PCNA expression in breast cancer

We did linear correlation analysis between AI and PCNA positive rate of neoadjuvant chemotherapy group and control group respectively. The correlation coefficient between AI and PCNA positive rate was r=−0.31 (P<0.05) in neoadjuvant chemotherapy group, and r=−0.46 in control group (P<0.05). It showed that AI and PCNA expression had negative correlation both before and after the chemotherapy (Fig.5).

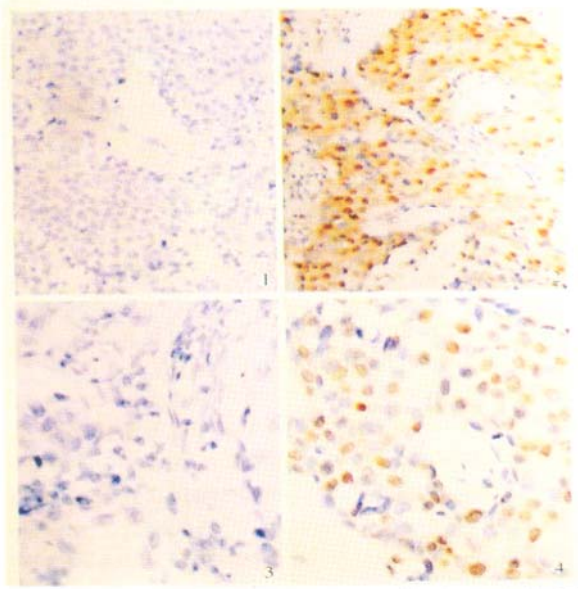

Fig. 1 Negative control of apoptosis cells by TUNEL(×200)
Fig. 2 Positively coloured apoptosis cells by TUNEL(×200)
Fig. 3 Negative control of PCNA(×200)
Fig. 4 Positively coloured PCNA cells by LSAB(×200)

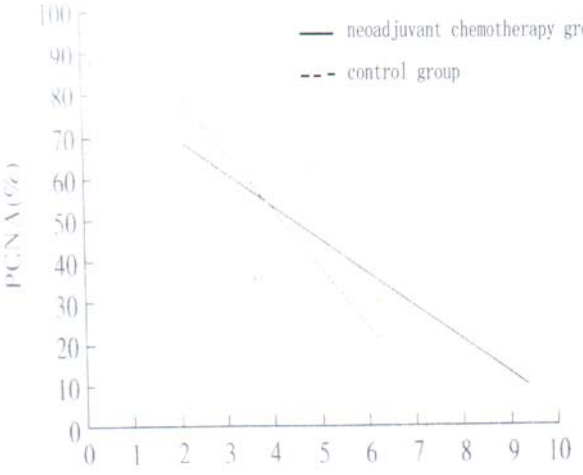

Fig. 5 Association between AI and PCNA

DISCUSSION

Affection of neoadjuvant chemotherapy to breast cancer cell apoptosis

Cell apoptosis is cell suicide in particular space-time. It is strictly controlled by organism. It is very important for human body to keep normal condition and functions[3]. Lack of cell apoptosis leads to cell [illegible] excess, and then form a tumor. Studies in lab have made sure that many chemotherapy drugs can induce tumor cell apoptosis by different action point[1,2]. In our study, AI of breast cancer had significant difference between neoadjuvant chemotherapy group and control group ($P<0.01$), and it's to say neoadjuvant chemotherapy can actually induce breast cancer cells apoptosis in human body.

Affection of neoadjuvant chemotherapy to breast cancer cell proliferation

Function of PCNA is to participate in synthesize and repair of DNA. Its expression can reflect the synthesize speed of DNA, and it's an important index to scale cell proliferation. Many scholars have taken the PCNA expression rate as one useful index to estimate the prognosis of breast cancer nowadays[4,5] In our study, PCNA expression rate of control group was highter than that of neoadjuvant chemotherapy group ($P<0.01$), which indicate that neoadjuvant chemotherapy can significantly reduce PCNA expression in breast cancer. It may point to that neoadjuvant chemotherapy can depress DNA synthesize speed of breast cancer cells, and so inhibit the proliferation of breast cancer.

Relation between AI and PCNA in breast cancer

AI and PCNA reflect tumor biological behavior from the point of death and proliferation respectively. Our study showed that AI and PCNA expression of breast cancer had negative correlation both before and after the neoadjuvanvt chemotherapy. It may indicate that breast cancer cells apoptosis and proliferation are interactional and restrict each other during the tumor progress process. It is reported that tumors with high AI and low PCNA expression will have better prognosis, and tumors with low AI and high PCNA expression will have worse prognosis[6,7]. So the neoadjuvant chemotherapy which can increase tumor cell apoptosis and reduce tumor cell PCNA expression may be useful for curing breast cancer.

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