Effects of Different Anticancer Drugs on the HEPG2 Cells Proteins Correlated with Cell Proliferation

Peng Li, Silang Zhou, Zhihong Chi, Jiren Zhang

Department of Neoplasm, Zhujiang Hospital, The southern medical university,Guangzhou, 510282, China

Abstract Objective To explore the variation of the proteins correlated with cells proliferation of HEPG2 (the human hepatoma cell lines) repeatedly treated by different anticancer drugs. **Methods** HEPG2 cells were repeatedly treated by anticancer drugs-doxorubicin, fluorouracil, cisplatin and combination of the three drugs. The expression of erbB-2 and c-myc in HEPG2 cells were tested by flow cytometry. The percentage of positive cells and the mean protein expression quantity of tumor cells were acquired by the fluorescence quantity analysis. Then we calculated the total quantity of protein of tumor cells in each group treated by different anticancer drugs. **Results** The total expression quantity of the proliferation-proteins in HEPG2 cells were unstable during cultivation. No correlation was found between any two groups of all 5 groups(4 groups treated respectively by doxorubicin、 etoposide、 cisplatin and combination of the three drugs and control group) in the total expression quantity of cells proliferation protein (P>0.05). **Conclusion** When HEPG2 cells were treated by different drugs or the same drug at different time, the proliferation protein of these cells was different, which suggested that it was necessary to develop individualized -chemotherapy.

Key Words flow cytometry; cells proliferation; tumor; cell line

It is a common thing that to apply same anticancer drugs for a few chemotherapy cycle time among clinic chemotherapy. It is a interesting problem to everyone of us that whether the biology characteristic of tumor cells, such as cells proliferation ability and so on, would have changes while they experience convention chemotherapy. But unfortunately, litter works had been done until recently .

In our study, HEPG2 (the human hepatoma cells lines) were repeatedly treated by anticancer drugs (Adriamycin, fluorouracil and cisplatin) which were used frequently in hepatoma chemotherapy. Then variation of the proliferation-proteins of HEPG2 when the cells were treated by different anticancer drugs.

MATERIALS AND METHODS

Cell Lines

HepG2 cells lines was purchased from Department of Neoplasm of the Nan Fang Hospital.

Main Reagents

Fetal calf serum, RPMI 1640 and parenzyme were purchased from Hyclone Ltd; antibody erbB-2 (mice)、antibody c-myc(mice) were purchased from Beijing Golden Bridge Biotechnology Co., Ltd; Adriamycin (ADM)、 fluorouraci (5-FU)and Cisplatin (DDP) were purchased from the Dispensary of Zhujiang Hospital.

Culture

HEPG2 The cells lines was maintained in RPMI 1640 medium and incubated at 37℃ is 5%-95% air with a high humidity. When cells proliferated to 50 bottle, these cells were grouped averagely to 5 groups as ADM group (cells were repeatedly treated by ADM)、5-FU group (cells were repeatedly treated by 5-FU)、 DDP group (cells were repeatedly treated by DDP)、combination group (cells were repeatedly treated by all anticancer drugs) and control group (cells were cultured routinely). The 3 anticancer drugs were all dilute by cell culture fluid.

The Concentration of The Anticancer Drugs

When cells proliferated logarithmly, HEPG2 cells were immigrated to cultivation plate with a cells density of 105 per milliliter and were cultured 4 hours. The concentration of the 3 anticancer drugs were measured by MTT method as concentration of these anticancer drugs to HEPG2 cells

were 20%, they were 0.11μg/ml(ADM), 1.6μg/ml (5-FU) and 0.86μg/ml (DDP) respectively.

When the time to subcultured, The corresponding anticancer drug were added to the HEPG2 cells of different groups according to half period of this drug.

Treatment Cycle

At begin, each group had 10 bottle cells. when cells of each group recovered from chemotherapy impairment and propagated to 10 bottle again, the cells can accept a new chemotherapy.

Flow Cytometry Test

Only one bottle of cells were cultured continuously and subcultured after every cells chemotherapy cycle. Cells in other 9 bottle were dissociated , washed for 2 times with PBS, fixed for 30 minutes with 0.5% paraformaldehyde at 4℃, perforated with 0.1% Triton, divided to 2 parts which were respectively added antibody of erbB-2 and c-myc, then were added with the second antibody after incubation, at last tested by flow cytometry. Every part was tested for about 3000 cells. The percentage of cells expressing these proteins and the mean quantity of them were acquired by the fluorescence quantity analysis.

Statistical Analysis

Every result was tested repeatedly for 2 times, the mean was selected as the final result. The total quantity of protein was the product of the mean quantity of protein and the percentage of cells expressing the proliferation proteins. The correlation of the total quantity of each protein between any 2 groups of total 5 groups was analyzed by spss10.0 software. $P<0.05$ was considered statistically significant.

RESULTS

The expression of erbB-2 protein

The expression of erbB-2 protein in control group HEPG2 cells was unstable during cultivation. The expression of erbB-2 protein in all groups cells was unstable when they were repeatedly treated by 3 anticancer for 6 cycles (Fig.1). The correlation of expression of erbB-2 protein in different cycles between different groups were analyzed by SPSS10.0 software, no correlation was found between any two groups of all 5 groups in the total quantity of cells proliferation protein except between ADM group and VP-16 group. The correlation coefficient were ranged from-0.398 to 0.772 ($P>0.05$). The correlation is statistical insignificance. The correlation coefficient between ADM group and VP-16 group is 0.919 ($P<0.05$) But the correlation is insignificance in statisticus when we applied a statistical way of partial correlation analysis, the correlation coefficient between ADM group and VP-16 group is 0.8725 ($P>0.05$).

The expression of c–myc protein

The expression of c-myc protein in control group HEPG2 cells was unstable during cultivation.

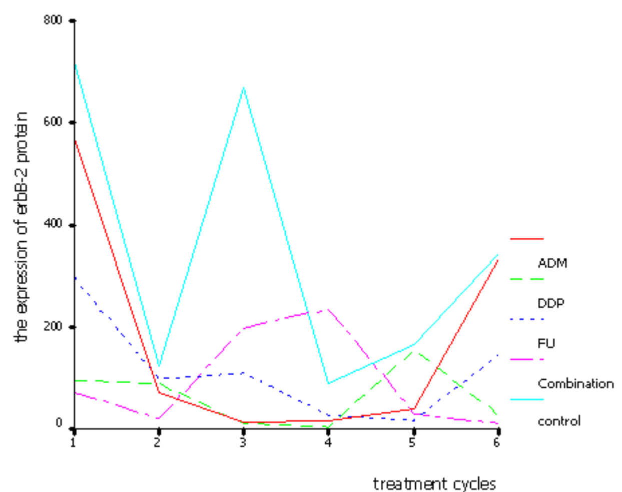

Fig.1 The expression of erbB-2 protein in HEPG2 cells treated with different anticancer drugs.

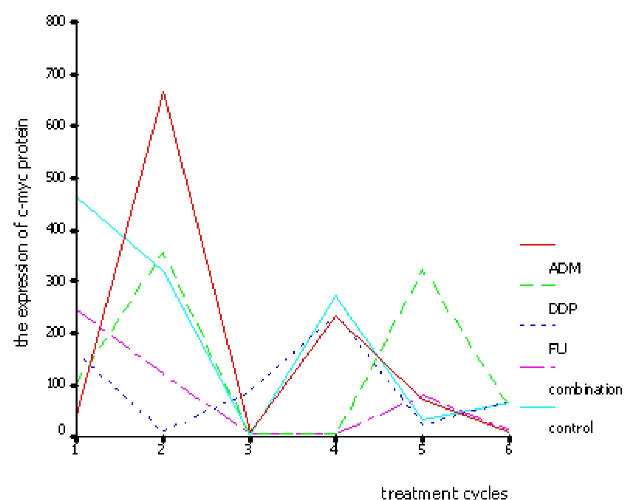

Fig.2 The expression of c-myc protein in HEPG2 cells treated with different anticancer drugs.

The expression of c-myc protein in all groups cells was unstable when they were repeatedly treated by 3 anticancer for 6 cycles (Fig. 2). The correlation of expression of c-myc protein in different cycles between different groups were analyzed by SPSS10.0 software, no correlation was found between any two groups of all 5 groups in the total quantity of cells proliferation protein. The correlation coefficient were ranged from-0.723 to 0.760 ($P>0.05$). The correlation is insignificance in statistics.

DISCUSSSION

Loss of cells growth control was the basic biological characteristic of the malignant tumor. The ability of cells proliferation can reflect the invasiveness of the malignant tumor. But it was always neglected that the effect of repeatedly chemotherapy on tumor cells.

erbB-2 and c-myc were oncogenes concerned with proliferation regulation of the tumor cells. So the expression of erbB-2 and c-myc can reflect the ability of cells proliferation. The function of erbB-2 included cells proliferation and cell differentiation. The over-expression of erbB-2 in tumor cells was a hint of highly ability of proliferation, powerfully invasiveness, easily metabasis and highly ability of chemoresistance [1,2]. If transmission pathway of erbB-2 had been blocked or monoclonal antibody neutralize the erbB-2 protein, the cell proliferation would be suppressed in the tumor cells in whom the expression of erbB-2 is positive. The apoptosis cells would be increase too [3,4]. The expression of c-myc in tumor cells had a tightly close relation with cells proliferation, cell differentiation and cells apoptosis [5,6]. Therefore, the cells proliferation and cells apoptosis could be suppressed by down-regulated the expression of c-myc[7].

For most solid tumor cells, especially for hepatoma cells, the sensitivity of anticancer were really low [8]. The reason of low sensitivity of anticancer drugs to solid tumor cells was in many ways such as proliferation, apoptosis and drug resistance of tumor cells[9]. In our study, we revealed the dynamic state regularity of erbB-2 and c-myc expression in HEPG2 cells when the cells were repeatedly treated by different anticancer drugs. Our findings were: (1)The expression of erbB-2 and c-myc of HEPG2 cells was unstable when the cells were cultured. It is to say that the biology characteristic of HEPG2 cells should be fugitiveness. (2) The expression of erbB-2 and c-myc of HEPG2 cells was unstable too when the cells were repeatedly treated by different anticancer drugs. (3) No correlation was found between the expression of erbB-2 and c-myc in HEPG2 cells from different groups. The results suggested that the biology characteristic of HEPG2 cells was unstable and indeterminate when the cells were treated by different anticancer drugs, or when the cells were treated by the same drug in different time, or even when the cells were normal cultured.

The clinic chemotherapy of a certain pathotype tumor based on the statistical results from clinic anticancer drugs sensitivity test [10]. A invariably chemotherapy way was always applied in the long–term chemotherapy procedure. At most time, the effects of tumor cells from chemotherapy were neglected. But our study indicated that, the expression of proliferation–proteins of tumor cells were unstable when the tumor cells were treated by different anticancer drugs. In clinic chemotherapy, it was a common thing that the expression of erbB-2 and c-myc was up-regulated or down-regulated. So we thought it might be ordinary that the biology characteristic of tumor cells always changed when the cells were treated by anticancer drugs in clinic chemotherapy. Unfortunately, this frequently changes of tumor cells biology characteristic were difficult to forecast. For this reason, the efficiency of chemotherapy in solid tumor was very low. It is thus evident that it is important to establish a precise system of individualization hemotherapy valuation in clinic tumor therapy. To adjust chemotherapy project according to the concrete biology characteristic of tumor cells is inevitable in future solid tumor chemotherapy.

REFERENCES

1. Nakazawa K, Dobashi Y, Suzuki S, et al. Amplification and overexpression of c-erbB-2, epidermal growth factor receptor, and c-met in biliary tract cancers. J Pathol. 2005, 206(3): 356–365.
2. Ma C, Lin H, Leonard SS, et al. Overexpression of erbB2 enhances ethanol–stimulated intracellular signaling and invasion of human mammary epithelial and breast cancer cells in vitro. Oncogene. 2003 Aug 14; 22(34): 5281–5290.
3. Leahy DJ. Structure and function of the epidermal growth factor (EGF/ErbB) family of receptors. Adv Protein Chem. 2004, 68:1–27.
4. Lu Y, Zi X, Zhao Y, et al. Insulin-like growth factor- I

receptor signaling and resistance to trastuzumab (Her-ceptin). J Natl Cancer Inst, 2001, 93(24): 1852–1857.

5. Donaldson TD, Duronio RJ. Cancer cell biology: Myc wins the competition. Curr Biol. 2004, 14(11): R425–7.
6. Hapke S, Kessler H, Luber B, and et al. Ovarian cancer cell proliferation and motility is induced by engagement of integrin alpha(v) beta3/Vitronectin interaction. Biol Chem. 2003, 384(7): 1073–1083.
7. Huang SG, Kong BH, Ma YY, et al. Impact of arsenic trioxide on proliferation and metastasis of drug–resistant human ovarian carcinoma cell line. Ai Zheng 2002, 21 (8): 863–867.
8. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. Hepatology. 2003, 37(2): 429–442.
9. Kim WR. The use of decision analytic models to inform clinical decision making in the management of hepatocel-lular carcinoma. Clin Liver Dis. 2005, 9(2): 225–34.
10. Awada A, Mano M, Hendlisz A, et al. New anticancer agents and therapeutic strategies in development for solid cancers: a clinical perspective. Expert Rev Anticancer Ther. 2004, 4(1): 53–60.