Effect of Trastuzumab in Combination with IFN α-2b on HER2 and MRP1 of ACHN

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Summary: To study the effect of Trastuzumab in combination with IFN α-2b on HER2 and MRP1 of ACHN in vitro, ACHN cell line of RCC was cultured by employing cell culture. The tetrazolium-based colorimetric assay was used to evaluate the growth-inhibiting effect of Trastuzumab with IFN α-2b. SP method was utilized to determine the expression of HER2 and MRP1 of the cells. Our results showed that Trastuzumab had inhibitory effect on the growth of renal tumor cells and reversing effect on the multi-drug resistance (MDR) in RCC in a time- and dose-dependent manner. Treated with Trastuzumab with or without IFN α-2b, the expression of HER2 and MRP1 genes of RCC was decreased significantly (P<0.05). It was concluded that Trastuzumab with IFN α-2b could inhibit the proliferation of RCC and the expression of HER2 and MRP1 of ACHN and to some extent, reverse the MDR of the tumor cells.

Key words: renal cell carcinoma; trastuzumab; IFN α-2b; HER2; MRP1

The incidence of renal tumors is second only to that of bladder tumors in male genitourinary system, and the tumors of kidney account for 2 %-3 % of tumors. The renal cell carcinoma (RCC) is a relatively common renal malignancy. Its treatment includes surgical and chemotherapeutic approaches, but neither of them is perfect. Drug resistance to chemotherapy developed by the tumor cells remains a major obstacle to the treatment. Thus, it is of great importance to find a method to reverse the drug resistance and to improve the curative effect. HER2, known as a factor of signal conduction, could induce the growth and drug resistance in tumor cells. This study, by using a special antibody to HER2, examined the curative effect and the possibility to reverse drug resistance of tumor cells.

1 MATERIALS AND METHODS

1.1 Materials
The ACHN cell line was bought from the Cell Storage Center, Wuhan University, China. Trastuzumab was purchased from Roche Co., Ltd., Switzerland. IFN α-2b was procured from Schering-Plough Co., USA. Rabbit anti human MRP1 was from Wuhan Boster Biological Technology Co., Ltd., China. Other reagents included HER2 (DACO Co., USA), MEM, trypsinase (Gibco, USA), MTT and DMSO (Sigma, USA) and calf serum (Hangzhou Sijiqing Biological Engineering Materials Co., Ltd., China).

1.2 Cell Incubation
The ACHN cell line was incubated in MEM culture solution containing 10 % calf serum, at 37 °C in 5 % CO2 in a culture box. The cells, after they grew and covered 80 % of the bottom of the culture bottle and adhered to the wall, were absorbed by 0.25 % trypsinase. When the space between the cells was enlarged and the cytoplasm shrunk, as observed under inverted microscope, the digestion was terminated and then the cells were incubated in culture bottles. As soon as the cells reached logarithm growing stage, the culture solution was discarded. Then the cells were rinsed with D-Hanks solution for examination.

1.3 Determination of Inhibition Rate
MTT method was used to determine the inhibition rate. After absorption with 0.25 % trypsinase, the concentration of the suspending solution was adjusted to 1 × 10⁴/ml. Then the cells were put into a 96-well plate, with 200 µl in each well, and were incubated overnight at 37 °C in 5 % CO2 in the culture box. Various chemotherapeutic drugs at different concentrations were added into the wells. 4 parallel wells were set up for each test, and the control wells contained 5 % ethanol solution. 48 h later, 10 µl MTT (5 mg/ml) was added into each well. After another 4 h, the supernatant was discarded. 200 µl DMSO was added into each well. By using ρ-Quant enzyme marking instrument, the A value of each well was determined at 490 nm. The inhibition rate was calculated by the following formula: Inhibition rate = (A value of control group - A value of experimental group)/A value of the control group × 100 %.

1.4 Immunohistochemical Determination of MRP1
The changes in HER2 and MRP1 were immunocytochemically evaluated. The ACHN cells, after being digested, well-mixed and adjusted to 1 × 10⁶/ml, were put into a 6-well plate with a coverslip for each well. When cells covered the entire cover-slip, the cells were rinsed with PBS and fixed with anhydrous ethanol and acetone. The expression of HER2 and MRP1 was evaluated by using SP method. For comparison, the known posi-
tive samples and PBS slices were used as positive and negative controls respectively. After the treatment of the cover slips, the first antibody (HER2, 1:200, MRP1, 1:200), the biotin-conjugated second antibody and SP reagent were added by following the instruction of kits. DAB kit was utilized for staining. The slips were subjected to routine dehydration, clearing and sealing. For each experimental group, 10 cells-coated coverslips were obtained. At least 10 fields of vision were observed or 500 cells were counted for each coverslip. The results were judged as follows: Stained HER2 and MRP1 were at plasma membrane, and the staining intensity was evaluated on a 4-degree scale—0 stands for no staining, 1 for yellowish staining, 2 for brown-yellow and 3 for brown. The expression of HER2 and MRP1 was expressed as positivity index, which was calculated by the formula: Positivity index=Total score/100 cells.

1.5 Administration of Drugs

In the experimental groups were added, IFN α-2b (250 U, 500 U, 1000 U, 2000 U, 5000 U, 10000 U), Trastuzumab (5 μg/ml, 10 μg/ml, 20 μg/ml, 40 μg/ml, 80 μg/ml, 120 μg/ml) or Trastuzumab plus IFN α-2b (40 μg/ml + 2000 U). For the control group, only 1 g/L DMSO solution was added.

1.6 Cross River Test

The vigorously growing ACHN cells were digested with 0.25 % trypsin and the digestion was terminated by washing with culture liquid. Then, ACHN cells suspension (3×10⁵/ml) was obtained by addition of MEM containing 10 % calf serum. The suspension was put into a 6-well plate, with 3 ml in each hole, and was incubated at 37 °C in 5 % CO₂ in the culture box for 24 h, to allow the cells to adhere to the wall. The culture liquid was discarded, and the culture solution containing Trastuzumab of different concentrations was added, with 3 ml in each well. In the control group, culture liquid of the same volume without drug was added. The samples were incubated for another 48 h at 37 °C in 5 % CO₂. Then the liquid was discarded. The cells were then rinsed with PBS. A straight line was set by a tip of 10 μl in each hole. Rinsed with PBS twice, the cells in the holes were immersed with MEM culture solution including 10 % calf serum and incubated at 37 °C with 5 % CO₂ in the culture box. The cells were observed once every 2 h until the straight lines were filled with growing cells. The same progress was repeated for 6 times.

All the data was analyzed by analysis of variance of SPSS 10.0 system. The prominent difference was recognized as P<0.05.

2 RESULTS

The inhibiting effect of Trastuzumab on ACHN cells was not remarkable at a lower concentration (5 μg/ml). The inhibiting effect was gradually obvious with the increase of the concentration. The rate was 58.59 % at the concentration of 80 μg/ml, shown in a dose-dependent manner, with the IC₅₀ of 46.4 μg/ml. IFNα-2b showed obvious killing-wound and inhibiting effect at lower concentration (500 U), of which the inhibiting rate was 99.36 % at the concentration of 5000 U, with the IC₅₀ of 1847 U (fig. 1).

The cells were inhibited obviously by Trastuzumab with IFN α-2b after 6 h. The inhibiting rate increased gradually with time past, which achieved 85.5 % at the time of 48 h. It was mainly time-effect and the most dependent time point was 48 h (fig. 2).

As above, the difference was prominent among the groups, and the killing-wound effect on ACHN cells was more obvious with the increase of drug concentration.

Treated with Trastuzumab, the cross river time of the cells in different groups was prolonged obviously. Compared with control group, the group of 10 μg/ml increased 25 %, the group of 80 μg/ml increased 160 %, while the group of over 120 μg/ml showed unobvious crossing after 72 h. The result showed that Trastuzumab could effectively inhibit the movement of ACHN cells (table 1).

HER2 and MRP1 of ACHN cells in control group showed mostly positive staining. The positive index was 0.628±0.032 for HER2 and 0.694±0.037 for MRP1. Treated plus Trastuzumab