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
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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 45.4 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).

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 % trypsinase 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.

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