Killing Effect of IL-13Rα2 Sensitized DC-CTL Cells on Mice Glioma in Vivo

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Abstract

To discuss the killing effect of IL-13Rα2 sensitized DC-CTL cells on mice glioma in vivo. Peripheral blood mononuclear cells (PBMC) of mice were isolated by Ficoll density gradient centrifugation and were cultured with rmGM-CSF, rmIL-4 and TNF-α into DCs in vitro. On the fifth day of being cultured by U251 cells, DCs was added with TNF-α and then co-cultured with the T cells from mice spleen to induce antigen-specificity CTL of U251 cells. Mice were given U251 cells by subcutaneous injection to prepare C57BL/6 glioma models, which were randomly divided into physiological saline (NS) group, DC group and IL-13Rα2-DC-CTL group. Weights of tumors, tumor volumes of mice in three groups were respectively measured on the ninth day of mice bearing tumors. Meanwhile, tumor growth curves were drawn and death cases of mice were observed. The average tumor volumes of mice respectively were 3.15 ± 0.3 cm³ (NS group), 2.97 ± 0.29 cm³ (DC group) and 2.21 ± 0.25 cm³ (IL-13Rα2-DC-CTL group) measured on the ninth day of bearing cancer, as the comparison among the tumor volumes of mice in three groups was statistically significant (p<0.001): the volume growth for IL-13Rα2-DC-CTL group was slower than that of the rest two groups (p<0.01); IL-13Rα2 antigen sensitized DC-CTL had a killing effect on glioma cells in vivo, which provided a new direction for the targeting immunotherapy of glioma.

Keywords — IL-13Ra2, Glioma, Immune targeted therapy

I. INTRODUCTION

Glioma is one of the cancers mostly found inside encephalic [1]. In recent years, although a certain curative effect has been gained with the advance of microsurgical techniques and development of comprehensive treatments like adjuvant chemotherapy and radiotherapy, the median survival time of glioma still hasn’t been obviously prolonged. IL-13Rα2’s specific expression on glioma, which doesn’t or rarely exists in normal tissues [2], has been described by vast amounts of literature and is found out to be in a positive correlation with the severity degree of tumors. Xiaobing J et al. adopted synthetic IL-13Ra2 antigen polypeptide sensitized CTL (cytotoxic lymphocyte) to specifically kill or wound glioma cells [3], and similar results were also obtained previously by our experiments. However, can IL-13Ra2 sensitized CTL specifically kill or wound glioma cells in vivo? This experiment will carry out research on this problem.

II. MATERIAL AND METHODS

A. The Subculture of U251 Cells

Took U251 cells out of storage tanks with liquid nitrogen and placed them in 37°C thermostatic water bath for fast rewarming; the rewarmed cells were scooped into culture flasks and were added with 5 ml cell culture fluid containing fetal calf serum, which would be changed every two or three days; part of U251 cells were detected with 0.4% trypan blue staining and cell viability was authenticated to be 95%; the cells were centrifuged in 1000 rpm for 5 minutes and then part of them were collected for subculture after washing twice; the cell concentration was 1x10⁶/ml and cells were collected and stored in cryogenic vials after centrifugation.

B. The preparation of U251 Cell Antigen and Dendritic cells (DCs)

Collected the U251 cells in logarithmic phase, then placed them in liquid nitrogen and 37°C water bath for multigelation, as the supernatant was
collected after the frozen-thawed cells being centrifuged. Finally, all crude antigens of tumors were extracted after filtration sterilization. Connective tissues around mice’s spleens were eliminated, cut the spleens into pieces and grinded them, then the cell suspension after filtration sterilization would be gathered. The centrifuged single-cell suspension of spleen was added with red cell lysate and placed under indoor temperature after blending, then the precipitated cells were collected and placed in a 37℃ incubator with CO₂ of 5% volume fraction.10ng/ml rmGM-CSF and 20ng/ml rmIL-4 were put into that and changed every two days. TNF-7 with 10ng/ml cell factors was refilled on the sixth day and the proliferative cell aggregations loosely adhesive to wall were percussed and the collected suspending cells were DCs on the eighth day.

C. Induced Culture of DC-CTL (CTL Sensitized by DCs)

Cultured those mature DCs and CTL in the proportion of 1 to 10 in 24-pore plates. The final concentration of DCs was 2×10³/ml and CTL’s was 2×10⁵/ml. The tumor antigen IL-13Rα2 was also added in and the final concentration was 20μg/ml. Fresh growth factors were refilled every other day and mature cells could be obtained three days later.

D. C57BL/6 mice’ Glioma Model

The experimental animals were bought from Shanghai Experimental Animal Center and Wenzhou Medical University’s Experimental Animal Center. All operations in the experiments and feeding were conducted according to the regulations on management and feeding of experimental animals in Wenzhou Medical University. Adjusted the cell concentration of all U251 to 5×10⁶/ml and injected it into mice subcutaneously to prepare glioma models. On the ninth day after injection, the tumor-bearing mice were randomly divided into three groups and 20 mice for each group, as the three groups were physiological saline(NS) group, DC group and IL-13Rα2-DC-CTL group. The method for the treatment was to give more subcutaneous injection around tumors. Provided the first treatment to all groups on the 9th day after implantation, the second treatment on the 15th day and third treatment on the 21st day after implantation. Then observed the tumor growth and survivals of mice once every three days. Calculated the tumor volumes according to the equation (V) = 1/2ab² (a means tumor’s long diameter and b is short diameter) and observed the death cases of mice; then figured out the survival rate. Stopped measuring volumes till the death rate of either group reached 50%. On the 21st day after implantation, all mice were killed. Tumors were fetched out and weighed, as the anti-tumor rate on tumor weight and volume were calculated respectively according to equations. Anti-tumor rate on weight = (1- average tumor weight of the treatment groups/average tumor weight of control group) × 100%. Anti-tumor rate on volume = (1- average tumor volume of the treatment groups/average tumor volume of control group) × 100%.

E. Statistical Analysis

Carried out statistics with SPSS17.0 statistical software. The data of every group was represented by mean ± standard deviation (x ± S) and one-way variance analysis was adopted on the comparison among those data of groups. Calculated survival rates with Ka. plan. Meier method. The difference was that P<0.05, which was statistically significant.

III. RESULTS

Implanted U251 cells in mice’s dorsum subcutaneously and tumor nodules appeared five days later. The tumors grew into mature ones (diameter >0.5cm) after implantation, as the average volume was about 0.2 cm³. After that, the tumor volumes grew rapidly. On the 18th day, the mean tumor volume of NS group was 3.15 ± 0.30cm³, DC group 2.97 ± 0.29 cm³ and IL-13Rα2-DC-CTL 2.21 ± 0.25 cm³. The comparison of tumor volume among these three groups had a remarkable statistical significance (Table.1, p<0.001). Compared with the data of last measurement, the tumor volumes of four mice in the IL-13Rα2-DC-CTL group were reduced
and the growth rate of other four mice’s tumors was also obviously slower than those in other groups. On the 20th day after implantation, the tumor volumes of mice in NS group grew obviously faster than that of other two groups, while the mean volume of IL-13Ra2-DC-CTL group was smaller than that of the other two groups (P<0.01). On the 21st day after implantation, the average tumor volume of three groups respectively were: 5.05±0.65cm³ for NS group, 3.49±0.60 cm³ for DC group and 1.51±1.07 cm³ for IL-13Ra2-DC-CTL group and the calculated anti-tumor rates of the groups were: 30.89% of DC group, 70.10% of IL-13Ra2-DC-CTL group while the average tumor weight of three groups respectively were 4.58±0.479g for NS group, 3.20±0.419g for DC group and 1.34±0.999g for IL-13Ra2-DC-CTL group and the calculated anti-tumor rate were: 30.13% of DC group, 70.74% of IL-13Ra2-DC-CTL group (P<0.01). The mean weight of tumors and mean volume of IL-13Ra2-DC-CTL group were both obviously smaller than those of the rest two groups (Table 2). It could be referred that more effective tumor suppression function showed up in the IL-13Ra2-DC-CTL group with increasing time of bearing the cancer.

### Table 1 The Tumor Volume in the Different Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor Volume(cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9d</td>
</tr>
<tr>
<td>NS</td>
<td>0.12±0.44</td>
</tr>
<tr>
<td>DC</td>
<td>0.13±0.46</td>
</tr>
<tr>
<td>IL-13Ra2-DC-CTL</td>
<td>0.13±0.44</td>
</tr>
</tbody>
</table>

### Table 2 The Volume and Weight Inhibition Rate in the Different group(21d)

<table>
<thead>
<tr>
<th>Group</th>
<th>Average Volume(cm³)</th>
<th>Rate of Volume inhibition (%)</th>
<th>Average Weight(g)</th>
<th>Rate of Weight inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>5.05±0.65</td>
<td></td>
<td>4.58±0.47</td>
<td></td>
</tr>
<tr>
<td>DC</td>
<td>3.49±0.60</td>
<td>30.89</td>
<td>3.20±0.41</td>
<td>30.13</td>
</tr>
<tr>
<td>IL-13Ra2-DC-CTL</td>
<td>1.51±1.07</td>
<td>70.10</td>
<td>1.34±0.99</td>
<td>70.74</td>
</tr>
</tbody>
</table>

### IV. CONCLUSIONS

IL-13Ra2’s specific expression on glioma, which doesn’t or rarely exists in normal tissues, making it possible that IL-13Ra2 can be a immune therapy target toward glioma. Here, we find that the mean weight and mean volume of tumors in IL-13Ra2-DC-CTL group were both obviously smaller than those of the rest two groups, so IL-13Ra2 antigen sensitized DC-CTL had a killing effect on glioma cells in vivo, which provided a new direction for the targeting immunotherapy of glioma.

### REFERENCES