Application of CAR-T Combined with Immunoassay Inhibitors in Tumor Treatment

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Abstract: Although some progress has been made in cancer treatment, there are still challenges such as tumor cell escape and recurrence after treatment. This article analyzed tumor surface antigens and their specificity, and designed antigen receptors for Chimeric Antigen Receptor T-Cell (CAR-T) targeting tumor cells. This article intended to use gene encoding techniques such as CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats-associated protein 9) to precisely regulate CAR-T cells and improve their therapeutic efficacy. On this basis, using in vitro amplification, culture and other methods, the large-scale production of CAR-T cells was achieved to ensure the sustained and stable therapeutic effect. This article tracked the therapeutic effects of mouse models and clinical trials on patients for a long time, and evaluated the persistence and stability of CAR-T combined with immunosuppressive agents. The survival time of CAR-T cells in vivo and their long-term inhibitory effect on tumors were analyzed. After using CAR-T treatment, the survival time of CAR-T cells was 120 days. The recurrence time of the tumor was 180 days, which was significantly longer than the 30 days in the tumor control group, indicating that CAR-T treatment has a certain effect on delaying tumor recurrence. This article comprehensively evaluated the efficacy and mechanism of CAR-T combined with immunosuppressive agents in tumor treatment, providing important scientific basis and experimental support for future clinical applications.

1. Introduction

With the rapid development of medical technology, the treatment of tumors has also undergone tremendous changes. Although traditional radiotherapy and chemotherapy can improve the survival rate of patients, there are also problems such as significant side effects and susceptibility to recurrence. Therefore, seeking more precise, efficient, and low side effect cancer diagnosis and treatment methods has become a focus of attention in the medical community. The emergence of CAR-T combined with immunosuppressive agents provides a new opportunity for the treatment of cancer.

This article first provided the exploration background of the application of CAR-T combined with immunosuppressive agents in tumor treatment, and pointed out some challenges that tumor treatment currently faces. Secondly, the application method of CAR-T combined with
immunosuppressive agents in tumor treatment was presented. Finally, the killing ability and persistence of CAR-T cells against tumor cells were evaluated through in vitro cell culture experiments. Flow cytometry and cell proliferation analysis were used to detect the immune activity and survival rate of CAR-T cells.

2. Related Work

The treatment of tumors is a multifaceted task that is related to the type, location, size, and physical condition of the patient. Myakoshina E B investigated clinical cases of ocular complications in the treatment of gastric stromal tumors [1]. Huan-Huan Wen believed that the treatment of malignant tumors is mainly based on modern medicine, and there may be adverse reactions after treatment [2]. Peng Z B believed that ribonucleotide reductase is a key enzyme in tumor proliferation [3]. Akakuru O U believed that accurate delineation of tumor boundaries is crucial for predicting cancer patient survival and evaluating the response of the tumor microenvironment to various treatment techniques such as chemotherapy and radiation therapy [4]. Du Y believed that hepatocellular carcinoma is a life-threatening disease, and there is currently no effective treatment method [5]. Cho H R used injectable hydrogel nanocomposites to maintain the permeability of brain tumors after surgery and continued administration [6]. Tao Xin conducted an in-depth analysis of the application of the rapid rehabilitation concept combined with traditional Chinese medicine nursing model in the treatment of gynecological malignant tumors [7]. Chen Jiawen explained the new strategy of using engineering bacteria to treat tumors and discussed some issues that need to be solved when using engineering bacteria to treat tumors in clinical practice [8]. Shan Bao'en believed that immune checkpoint inhibitors can restart and maintain the tumor immune cycle, normalizing the anti-tumor immune response [9]. He Yiman believed that abnormal energy metabolism is one of the typical characteristics of tumors, and most tumors highly rely on aerobic glycolysis to quickly obtain energy [10]. However, their research is not very effective in treating tumors.

CAR-T therapy is a novel and precise treatment method that utilizes genetic engineering to specifically recognize and kill T lymphocytes. This treatment method has high targeting and precise killing of tumor cells, with low toxicity and side effects, and is highly favored by patients and physicians. Immunoassay inhibitors are drugs that regulate the body's immune function and enhance anti-tumor immune response. This method can prevent cancer cells from escaping the body's immune response, allowing the body's immune system to more efficiently recognize and kill tumor cells. The combination of CAR-T therapy and immune checkpoint inhibitors can achieve synergistic effects between the two. CAR-T cells can target and kill tumor cells, while immune checkpoint inhibitors can enhance the body's immune response. The combination of the two can effectively control tumors.

3. Methods

3.1 CAR-T Cell Design and Construction Optimization

Step 1: Tumor surface antigens and their specificity are analyzed, and antigen receptors for CAR-T cells are designed with tumor cells as targets.

Step 2: CAR is structurally optimized, including the regulation of antigenic receptor affinity and signal transduction modules, to enhance the activity of CAR-T cells.

Step 3: This article intends to use gene coding techniques such as CRISPR-Cas9 to precisely regulate CAR-T cells and improve the therapeutic effect of CAR-T cells.

Step 4: On this basis, using in vitro amplification, culture and other methods, the large-scale
production of CAR-T cells is achieved to ensure the sustained and stable therapeutic effect.

The formula for the degree of signal pathway activation $\text{Pathway Activation}_\text{Level}$:

$$\text{Pathway Activation}_\text{Level} = \text{Expression}_\text{After} - \text{Expression}_\text{Before} \sum (\text{Weight}_i + \text{Molecule}_i) \tag{1}$$

Among them, $\text{Molecule}_i$ is the activity or expression level of the $i$-th key molecule in the signaling pathway, and $\text{Weight}_i$ is the weight of the molecule's contribution to the activation of the signaling pathway. $\text{Expression}_\text{Before}$ and $\text{Expression}_\text{After}$ represent pre expression and post expression, respectively.

3.2 Application of In Vitro Amplification and Culture Techniques to Immunosuppressive Agents

Step 1: Immunoassay inhibitor screening: PD-1 (Programmed Death 1), CTLA-4 (cytotoxic T lymphocyte-associated antigen-4) antibodies, as well as other immune regulatory factors.

Step 2: This article aims to optimize the dosage, route, and duration of administration to maximize its anti-tumor effects.

Step 3: On this basis, by using bioinformatics and other methods, its role in the tumor microenvironment is predicted, providing new ideas for personalized diagnosis and treatment [11-12].

Step 4: This article intends to explore the anti-tumor immune escape effects and mechanisms of immune checkpoint inhibitors from both in vivo and in vitro perspectives through methods such as cellular immunology and molecular biology, and explore their possible targets of action.

The formula of immune resistance comprehensive score $\text{Immunoresistance}_\text{Score}$:

$$\text{Immunoresistance}_\text{Score} = K_i \int S_i \tag{2}$$

Among them, $K_i$ is the $i$-th factor that affects immune resistance (such as gene expression, signal pathway activation, etc.), and $S_i$ is the coefficient of this factor.

Immune tolerance prediction model $\text{MN}_Y$:

$$\text{MN}_Y = H_0 + H_1 Y_1 + H_2 Y_2 + \cdots + H_k Y_k \tag{3}$$

Among them, $H_0$ to $H_k$ are regression coefficients, and $Y_1$ to $Y_k$ are multiple factors that affect immune tolerance.

4. Results and Discussion

4.1 Immunoresistance Experiment of CAR-T Cells

The killing ability and persistence of CAR-T cells against tumor cells were evaluated through in vitro cell culture experiments. Flow cytometry and cell proliferation analysis were used to detect the immune activity and survival rate of CAR-T cells.

Experimental conditions: Different conditions used in the experiment were described, such as the formulation of the culture medium, pre-treatment of CAR-T cells, etc.

Tumor cell type: It represents the different types of tumor cells used in the experiment.

Condition A:

The CAR-T cell killing rate of tumor type 1 was 85%, with a persistence of 14 days. The CAR-T cell killing rate of tumor type 2 was 78%, with a persistence of 12 days.

Condition B:

The CAR-T cell killing rate of tumor type 1 was 92%, with a persistence of 18 days. The CAR-T cell killing rate of tumor type 2 was 83%, with a persistence of 15 days.
Condition C:
The CAR-T cell killing rate of tumor type 1 was 95%, with a persistence of 21 days. The CAR-T cell killing rate of tumor type 2 was 87%, with a persistence of 17 days. The immune resistance test results of CAR-T cells are shown in Table 1.

Table 1: Results of immune resistance experiments on CAR-T cells

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Tumor cell type</th>
<th>CAR-T cell count</th>
<th>Killing rate (%)</th>
<th>Persistence (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition A</td>
<td>Tumor type 1</td>
<td>1x10^6</td>
<td>85</td>
<td>14</td>
</tr>
<tr>
<td>Condition A</td>
<td>Tumor type 2</td>
<td>1x10^6</td>
<td>78</td>
<td>12</td>
</tr>
<tr>
<td>Condition B</td>
<td>Tumor type 1</td>
<td>2x10^6</td>
<td>92</td>
<td>18</td>
</tr>
<tr>
<td>Condition B</td>
<td>Tumor type 2</td>
<td>2x10^6</td>
<td>83</td>
<td>15</td>
</tr>
<tr>
<td>Condition C</td>
<td>Tumor type 1</td>
<td>3x10^6</td>
<td>95</td>
<td>21</td>
</tr>
<tr>
<td>Condition C</td>
<td>Tumor type 2</td>
<td>3x10^6</td>
<td>87</td>
<td>17</td>
</tr>
</tbody>
</table>

4.2 Experiments on the Inhibitory Effect of Combination Therapy on Tumors

A mouse tumor model was constructed to evaluate the efficacy of CAR-T combined with immunosuppressive agents in treating tumors in vivo. Tumor volume measurement and histological analysis were used to evaluate the inhibitory effect of combination therapy on tumor growth and metastasis.

The experimental results of the inhibitory effect of combination therapy on tumors are shown in Figure 1. Scheme 1 is a control group+tumor type A; Scheme 2 is CAR-T group+tumor type A; Scheme 3 is an immune test inhibitor group+tumor type A; Scheme 4 is a combination therapy group with tumor type A; Scheme 5 is a control group+tumor type B; Scheme 6 is CAR-T group+tumor type B; Scheme 7 is an immunosuppressive test group+tumor type B; Scheme 8 is a combination therapy group with tumor type B.

Figure 1 (a) shows the initial tumor volume and the tumor volume at the end of treatment. Initial tumor volume:
The initial tumor volume for schemes 1, 2, 3, and 4 was 100mm³.
The initial tumor volume for schemes 5, 6, 7, and 8 was 120mm³.
End of treatment tumor volume:
The tumor volume of Scheme 1 increased from 100mm³ to 400mm³.
The tumor volume of Scheme 4 remains unchanged at the end of treatment, at 100mm³.
The tumor volume of Scheme 8 did not increase at the end of treatment, reaching 120mm³.

Figure 1 (b) shows the tumor growth inhibition rate and metastasis rate. Tumor growth inhibition rate:
Scheme 4 had the highest tumor growth inhibition rate, at 75%.
The growth inhibition rate of Scheme 2 was 50%, which was relatively high.
The tumor growth inhibition rate of Scheme 1 was 0%, indicating that the tumor continued to grow during treatment.
The growth inhibition rate of Scheme 5 was also 0%, and the initial volume and treatment end
volume were relatively large.

Transfer rate:

Scheme 1 had the highest transfer rate of 60%.
The transfer rate of Scheme 5 was 55%, ranking second.

Figure 1: Experimental results of the inhibitory effect of combination therapy on tumors

Scheme 4 performed the best in tumor growth inhibition and had the lowest metastasis rate, indicating that this treatment scheme is more effective in controlling tumor growth and preventing metastasis.

Scheme 1 performed the worst in tumor growth inhibition and had the highest metastasis rate, which may indicate that this treatment scheme is not effective for specific tumor types or patient populations.

There seems to be no significant direct correlation between tumor growth inhibition rate and metastasis rate, and some schemes have relatively low metastasis rates even if the growth inhibition rate is not high (such as Scheme 2). The treatment and metastasis mechanisms of tumors may involve multiple complex factors that need to be comprehensively considered.

4.3 Immunotolerance Experiment

The immune tolerance of patients to CAR-T cell therapy was evaluated through mouse models and clinical trials. The levels of cytokines and the expression of immune related genes in the patient's serum were analyzed, and the immune side effects of CAR-T treatment were explored. Scheme 1 is a healthy control group+no treatment; Scheme 2 is a tumor control group+no treatment; Scheme 3 is CAR-T group+CAR-T treatment; Scheme 4 is an immunoassay inhibitor group+immunoassay inhibitor treatment; Scheme 5 is a combination therapy group+CAR-T combined with immunosuppressive agents. The results of the immune tolerance experiment are shown in Figure 2.

Scheme 1 had the lowest cytokine level at 50 pg/mL. Scheme 3 had the highest cytokine level, reaching 300 pg/mL. The cytokine levels of Schemes 2, 4, and 5 ranged from 180 pg/mL to 220 pg/mL.

There are significant differences in cytokine levels among different regimens, which may reflect the varying degrees of immune system stimulation or inhibition by different regimens. Cytokines are important regulatory factors in the immune system, and changes in their levels may directly affect the intensity of the immune response.
Figure 2: Results of immune tolerance experiment

For Figure 2, cytokine levels (pg/mL).

4.4 Experiments on the Reduction of Side Effects of Immunosuppressive Agents

Lymphocyte proportion (%):

The proportion of lymphocytes in Scheme 1 was 25%, which was the lowest. Scheme 4 had the highest proportion of lymphocytes, at 40%. The proportion of lymphocytes in Scheme 2 and Scheme 3 was 30% and 35%, respectively.

The change in lymphocyte ratio can reflect different states of the immune system. The increase in lymphocyte proportion is usually related to immune response or viral infection. Scheme 4 had the highest proportion of lymphocytes, which may indicate that it stimulates a strong immune response.

The experimental results of reducing the degree of side effects of immunosuppressive agents are shown in Figure 3.

Figure 3: Experimental results of reducing the degree of side effects of immunosuppressive agents
Scheme 4 showed good results in white blood cell count, lymphocyte ratio, and inflammation marker levels, which may indicate that the plan is more effective in activating immune responses and controlling inflammation. Meanwhile, Scheme 4 had the lowest side effect score, indicating a high level of safety.

It is possible to conduct in-depth research on the differences in lymphocyte count, lymphocyte ratio, inflammatory indicators, and their correlation with adverse reactions between two groups of patients. Meanwhile, it is also possible to consider combining other clinical indicators and patient feedback to comprehensively evaluate the effectiveness and safety of different regimens.

### 4.5 Persistence Experiment of Immunotherapy Effect

The persistence and stability of CAR-T combined with immunosuppressive agents were evaluated by long-term tracking of therapeutic effects in mouse models and clinical trials of patients. The survival time of CAR-T cells in vivo and their long-term inhibitory effect on tumors were analyzed.

#### Healthy control group:

There is no treatment in this group, so the data for CAR-T cell survival time, tumor recurrence time, and long-term inhibition rate are all "-", indicating that no relevant measurements or evaluations have been conducted.

#### Tumor control group:

The group also had no treatment, so the data for CAR-T cell survival time is "-". The tumor recurrence time was 30 days, indicating that the rate of tumor recurrence is faster without treatment.

The long-term inhibition rate was 0%, indicating that the tumor has not been effectively controlled in the long term without treatment.

<table>
<thead>
<tr>
<th>Table 2: Persistent experimental results of immunotherapy efficacy</th>
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<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>Healthy control group</td>
</tr>
<tr>
<td>Tumor control group</td>
</tr>
<tr>
<td>CAR-T group</td>
</tr>
<tr>
<td>Immunoassay inhibitor group</td>
</tr>
<tr>
<td>Combination therapy group</td>
</tr>
<tr>
<td><strong>CAR-T group:</strong></td>
</tr>
</tbody>
</table>
Combination therapy group:

After combined use of CAR-T and immunosuppressive agents, the survival time of CAR-T cells reached 180 days, which was longer than the 120 days treated with CAR-T alone, indicating that combined treatment may help prolong the survival of CAR-T cells. The tumor recurrence time was 270 days, which was the longest among all groups, indicating that combination therapy had the best effect in delaying tumor recurrence. The long-term inhibition rate reached 85%, significantly higher than the other groups, indicating that combination therapy had a very significant effect on long-term tumor control. The results of the persistence experiment on the efficacy of immunotherapy are shown in Table 2.

In summary, from the two key indicators of tumor recurrence time and long-term inhibition rate, the combined use of CAR-T and immunosuppressive agents showed the best therapeutic effect. However, it should be noted that these results may be influenced by various factors, such as individual differences in patients, tumor types, and the specific implementation of treatment plans.

5. Conclusions

This article took mice as the research object and explored their effects on immune function through in vivo experiments. The previous research has shown that CAR-T combined with immunoassay has the lowest adverse reaction score, indicating high safety and good patient tolerance. Individual differences in patients, types of tumors, specificity of treatment plans, and potential adverse reactions are all necessary. In addition, if the patient has a larger tumor in the early stages, they should be closely monitored and more aggressive therapies should be taken to reduce the metastasis of cancer cells. This article is just an immunotherapy plan, and in the future, more tumor treatment plans can be considered for development.

References