Clinical research on dendritic cell vaccines to prevent postoperative recurrence and metastasis of liver cancer

T.Y. Sun, W. Yan, C.M. Yang, L.F. Zhang, H.L. Tang, Y. Chen, H.X. Hu and X. Wei

General Surgery Department, Air Force General Hospital, Beijing, China

Corresponding author: X. Wei
E-mail: suntiye_ty@163.com

ABSTRACT. We aimed to evaluate dendritic cell (DC) tumor vaccines for preventing liver cancer recurrence and metastasis. DCs were induced from mononuclear cells in the peripheral blood with recombinant human granulocyte macrophage colony stimulating factor (rhGM-CSF) and recombinant human interleukin 4 (rhIL-4), followed by sensitization with lysis of autologous liver cancer cells. One hundred and sixty patients with hepatocellular carcinoma were randomly divided into two groups of 80. One group was treated postoperatively with six cycles of the DC tumor vaccine. The other group was treated postoperatively with six cycles of FOLFOX 6, beginning 1 week after surgery. After treatment with DC tumor vaccines, the levels of CD3+, CD4+, and CD8+, the ratio of CD4+ to CD8+ DC, and the serum levels of IL-12 and IFN-γ were significantly increased both in comparison to the pre-treatment levels ($P < 0.001$) and to the chemotherapy group ($P < 0.001$). After a postoperative follow-up of 18 months, the metastatic recurrence rate in the DC tumor vaccine group was significantly lower than that in the chemotherapy group (17.50 vs 48.75%, $P < 0.005$), and the survival rate of the patients in the DC tumor vaccine group was higher than that of the chemotherapy treatment group (86.25 vs 52.50%, $P < 0.005$). Treatment with DC tumor vaccines was safe and

feasible. It can enhance the immunity of the patients, reduce the rates of metastasis and recurrence, and improve survival rates. This is a promising treatment for the prevention of postoperative recurrence in patients with liver cancer.

Key words: Liver; Dendritic cells; Immune treatment; Chemical treatment; Recurrence; Metastasis

INTRODUCTION

Liver cancer is currently the fifth most common malignancy worldwide. Although radical resection is the most effective treatment for primary liver cancer, 60-70% of patients relapse within 5 years, which leads to a high local recurrence rate (Banchereau et al., 2001). Therefore, a positive and effective method to prevent postoperative recurrence is needed, and this is an active area of research. In a recent study, dendritic cells (DCs) were discovered to be the most powerful antigen-presenting cells (APC) in the body's immune system, playing a key role in initiating and controlling the immune response (Yamanaka et al., 2005; Perambakam et al., 2006).

Our previous studies have shown that DCs can effectively induce resistance to gastric cancer (Sun et al., 2010a), and in recent years, a tumor vaccine based on DCs has been effective in the treatment of melanoma (Banchereau et al., 2001). However, the study of prevention of postoperative recurrence and metastasis in patients with hepatocellular carcinoma is still at the exploration stage.

Therefore, we conducted a randomized controlled trial comparing a DC tumor vaccine to chemotherapy. The outcomes of interest were recurrence rates, survival, and immune functions.

Therefore, this study recruited 160 patients with liver cancer who met the inclusion criteria and underwent curative resection at the Department of Hepatobiliary Surgery at our hospital from July 2008 to July 2011. The cases were randomly divided into two groups, one to be treated with DC tumor vaccines and the other, the chemical treatment group, to be treated with chemotherapy.

Peripheral blood was extracted from the patients in the DC tumor vaccine group and then the DCs were induced from the peripheral blood mononuclear cells (PBMC) by stimulation with recombinant human granulocyte macrophage colony stimulating factor (rhGM-CSF), recombinant human interleukin (rhIL-4), and other activating cytokines. The DCs were primed by injecting autologous liver cancer cell lysis into the patient's body 1 week later. We also discuss the immune function of DC tumor vaccines sensitized by autologous liver cancer cell lysis to stimulate the body to resist liver cancer, a new technique for postoperative biological immunotherapy of liver cancer patients.

MATERIAL AND METHODS

Clinical data

We recruited 160 patients with liver cancer who underwent curative resection in the Department of Hepatobiliary Surgery at our hospital from July 2008 to July 2011 for our randomized controlled study. All of the patients had resected metastases or were without distant metastases, none had serious viscera insufficiency, and they were all diagnosed with hepatocellular carcinoma by histologic examination postoperatively.

The patients were divided into two groups at random using a digital method. Eighty cases were assigned to the chemical treatment group, 56 men and 24 women, with an average age of 53.2 ± 7.5 years. After surgery, they underwent chemical treatment according to the standard chemotherapy regimen FOLFOX 6.

Eighty cases were assigned to the DC tumor vaccine treatment group, 56 men and 24 women with an average age of 57.3 ± 7.1 years. After surgery, these patients were given six cycles of DC tumor vaccine treatment.

The two groups were comparable in age, gender, alpha-fetoprotein (AFP) levels, tumor size, tumor differentiation degree, the degree of liver cirrhosis, and whether the cancer was infringing on the vascular system (P > 0.05).

This randomized controlled trial was approved by the medical Ethics Committee. All of the patients and their families signed the informed consent.

All patients were not receiving systemic chemotherapy. The inclusion criteria were: 1) a single tumor; 2) no preoperative blood transfusion; 3) the pathological diagnosis was hepatocellular carcinoma; 4) the preoperative examination found no distant metastases; 5) the cut edge of the tumor was larger than 1 cm; 6) the Karnofsky score was greater than 70; 7) the patients were compliant with treatment and could be followed up easily. There were no other specific exclusion criteria.

Main reagents and instruments

AIM-V serum-free medium was provided by the Gibco company. Ficoll lymphocyte separation medium was provided by the Lymphoprep company. rhGM-CSF, rhIL-4, and tumor necrosis factor (TNF)-α were provided by the PeproTech company. Fluorescence-tagged (FITC) monoclonal antibodies, including cD3-Tc, IL-4-PE, and IFN-γ were provided by the Caltag company; CD8-FITC and CD4-PE were provided by the BD Pharmingen company. The FACS Calibur flow cytometry (FCM) instrument was provided by the BD company.

Separation of PBMC

Patients were injected subcutaneously with 100 μg recombinant rhGM-CSF every day for mobilization of monocytes. After 5 days, 100 mL fresh peripheral blood with anticoagulation was collected. PBMC were separated by Ficoll density gradient centrifugation.

Preparation for freeze-thaw lysis of the liver tumor

The tumor tissue was cut up and placed in liquid nitrogen for 10 min, followed by a water bath at 37°C. The freeze-thaw cycle was repeated three times. The tissue was then further broken open with an ultrasonic instrument. The released fluid was collected by centrifugation and filtering through a 0.22-μm microporous membrane. The total protein content was measured by the Biuret method, and the protein concentration was adjusted to 1.0 g/L.

Preparation of DC tumor vaccines

The PBMC were collected and washed twice, and adjusted to 5×10^6 cells per milliliter. They were placed in a culture bottle at 37°C with 5% CO_2 for 2 h. The non-adherent cells were

washed out and the adherent cells were cultivated in RPMI 1640 media including rhGM-CSF (100 µg/L) and IL-4 (100 µg/L) for 1 day, after which the nutrient solution was replaced and cytokines were added. On the fifth day, the liver lysis antigens (100 µg/mL) and TNF-α (100 µg/mL) were added. The tumor vaccines were collected on the eighth day.

Chemotherapy treatment

The chemical treatment group was given chemotherapy 1 week after surgery with the FOLFOX 6 chemotherapy regimen described below:

Oxaliplatin at 100 mg/m^2 was added in 500 mL glucose at a ratio of 5%, and it was administered by intravenous drip over 180 min on the first day.

Leucovorin calcium at 200 mg/m^2 in 250 mL saline was also administered by intravenous drip over 120 min on the first day.

Fluorouracil at 400 mg/m^2 was administered by intravenous injection, and then a dose of 2400 mg/m^2 was administered by a trace vein pump over 46 h.

Five milligrams of tropane SiQiong was injected 30 min before chemotherapy in order to prevent patients from having digestive tract reactions such as nausea and vomiting. The treatment was repeated every 2 weeks, and 4 weeks was counted as one cycle. The treatment was discontinued in cases of disease progression, intolerance, or toxicity. The longest treatment cycle was 6 weeks.

Before and after each cycle of chemotherapy, the urine was tested for blood, and liver and kidney function, fasting blood glucose, blood AFP, and electrocardiogram information was collected. The curative effect was evaluated after two courses of treatment and at the 18-month follow-up time.

DC tumor vaccines

In the DC tumor vaccine treatment group, DC tumor vaccine treatment was administered 1 week after surgery. The vaccine was injected intradermally in the upper limbs, alternating between sides, with a dose of 8.9 x 10^9 cells per injection. An injection was given once a month, and each month was counted as one cycle of treatment. Six cycles of treatment were given in total. After each injection, patients were observed for adverse reactions such as fever and late-onset hypersensitivity.

Observation method

Detection of immunological indexes

Before and after the treatment with DC tumor vaccines, the T lymphocyte subgroup such as CD3+, CD4+ and CD8+ was detected in the peripheral blood by FCM. Five milliliters of peripheral blood with heparin anticoagulant was mixed with 10 µL of the corresponding fluorescence-labeled antibody, and then incubated at 4°C for 20 min. Then, 2 mL of the pyrolysis liquid was added, and it was placed at room temperature protected from light for 10 min. The supernatant was discarded after centrifuging and the cells were washed twice with PBS. After fixing in 1% paraformaldehyde, the

labeled cells were detected by FCM and the data were analyzed with the Cell Quest software. The positive expression rate of the obtained sample was 1×10^4, and the ones tagged with fluorescence of the same type of IgG as the corresponding negative control.

The levels of IL-10, IL-12, and IFN-γ in the serum were detected by an enzyme-linked immunosorbent assay. The concentration of peripheral blood cells was adjusted to 5×10^4/mL, which was placed in six wells. After 4 h of culturing, we tested the levels of IL-10, IL-12 and IFN-γ in the cell supernatant.

Clinical observation and follow-up

Clinical evaluation was performed 1 week before the start of treatment, the 3rd week during the treatment, and 3 weeks after the treatment. The evaluation included a comprehensive collection of medical history, physical examination, routine blood tests, liver and kidney function tests, carcinoembryonic antigen levels, AFP levels, a total abdominal ultrasound, and a chest X-ray examination. When the patients were suspected of having a tumor recurrence or metastasis, abdominal computed tomography (CT) or magnetic resonance imaging (MRI) and puncture biopsy were performed. Every 3 months after surgery, the patients had serum levels of AFP, IL-10, IL-12, IFN-γ measured, and they underwent an abdominal ultrasound, CT or MRI, and a chest X-ray. All patients were followed up until January 2013 or to the date of death.

Statistical analysis

The SPSS 16.0 statistical software was used to conduct data analysis. Results are reported as means ± standard deviation. Chi-squared tests and *t*-tests were applied for group comparisons. All statistics were two-sided, with the level of significance set at α = 0.05. Therefore, a P < 0.05 means that the difference was statistically significant, and a P < 0.01 means that the difference was even more significant.

RESULTS

Clinical observations of the DC tumor vaccine application

The average number of DC induced in 100 mL peripheral blood was 9.2×10^{10}. The number of DC obtained was measured by FCM using the mature expression phenotype of DC (CD1a, CD83, CD86, CD80, CD40, and HLA-DR), (Table 1 and Figure 1).

Table 1. Expression rate of cell surface marker in patients before and after treatment in the two groups (%, means ± SD).

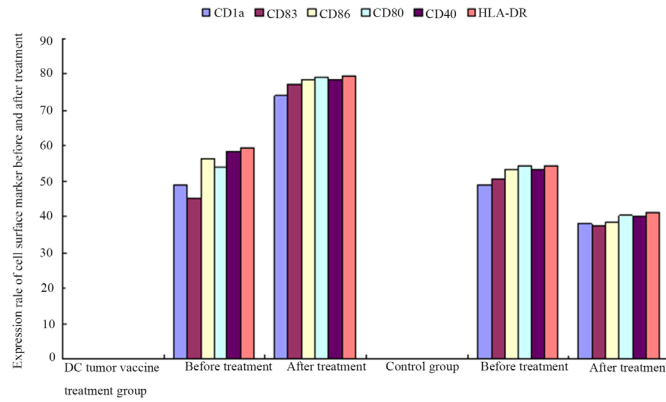|	CD1a	CD83	CD86	CD80	CD40	HLA-DR
DC tumor vaccine treatment group						
Before treatment	49.05 ± 3. 27	45.11 ± 3.10	56.33 ± 3.54	54.15 ± 2.76	58.39 ± 2.83	59.45 ± 3.61
After treatment	74.12 ± 3.29	77.27 ± 3.15	78.51 ± 3.48	79.19 ± 2.81	78.44 ± 2.87	79.42 ± 3.57
Control group						
Before treatment	49.13 ± 2.22	50.43 ± 2.52	53.34 ± 3.56	54.28 ± 2.32	53.56 ± 2.47	54.27 ± 3.34
After treatment	38.21 ± 2.33	37.48 ± 2.61	38.55 ± 3.49	40.31 ± 2.42	40.14 ± 2.51	41.19 ± 3.29

Figure 1. Expression rate of cell surface markers before and after treatment in the two groups (%, means ± SD).

Changes of T lymphocyte subsets before and after treatment in the two groups

The levels of CD3+, CD4+, and CD4+/CD8+ in the peripheral blood of the DC tumor vaccine treatment group increased significantly relative to before treatment (P < 0.001), and the post-treatment levels were also significantly higher than that of the chemical group after treatment (P < 0.001) (Table 2 and Figure 2).

Table 2. Changes of T lymphocyte subsets before and after treatment in the two groups (% ± SD).

Groups	CD3+	CD4+	CD8+	CD4+/CD8+
DC tumor vaccine treatment group				
Before treatment	50.33 ± 5.82	39.56 ± 7.29	35.64 ± 6.23	0.97 ± 0.42
After treatment	73.29 ± 6.65*	67.62 ± 8.14*	66.37 ± 7.42*	1.68 ± 0.59
Control group				
Before treatment	52.14 ± 4.37	38.46 ± 5.11	33.55 ± 4.72	1.21 ± 0.53
After treatment	46.17 ± 5.22	34.39 ± 6.02	31.23 ± 5.52	1.13 ± 0.76

*Means compared with the DC tumor vaccine treatment group before treatment, t = 3.56, 4.62, 5.87, P values <0.001, Compared with the control group after treatment, t = 3.27, 4.83, 5.72, P values <0.001 to 0.01.
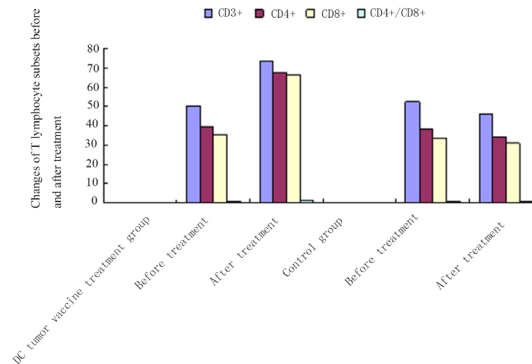

Figure 2. Changes of T lymphocyte subsets before and after treatment in the two groups (%, means ± SD).

Changes of of IL-10, IL-12 and IFN-γ levels in the serum in the two groups

The concentration of IL-10 in the serum was 136.11 ± 16.53 ng/L in the DC tumor vaccine treatment group after treatment, which was significantly lower than that before treatment (375.45 ± 17.34 ng/L, P < 0.01). The concentration of IL- 2 and IFN-γ in the serum of the DC tumor vaccine treatment group after treatment was 1083.43 ± 54.67 and 1079.25 ± 54.74 ng/L, respectively, which was significantly higher than that before treatment (435.31 ± 16.44 and 425.13 ± 17.05 ng/L, respectively, P < 0.001), and was also significantly higher than that in the chemical group after treatment (516.41 ± 18.58 and 531.38 ± 19.08 ng/L, respectively, P < 0.01) (see Table 3 and Figure 3).

Table 3. Changes of three kinds of cytokines before and after treatment in the two groups (%, ± SD).

Groups	IL-10 (ng/L)	IL-12 (ng/L)	IFN-γ (ng/L)
DC tumor vaccines treatment group			
Before treatment	428.37 ± 12.15	526.38 ± 13.27	539.28 ± 13.14
After treatment	129.28 ± 12.22*	1265.41 ± 62.34*	1312.34 ± 63.38*
Control group			
Before treatment	394.26 ± 12.39	497.35 ± 13.28	542.59 ± 13.82
After treatment	305.29 ± 13.11	601.39 ± 25.72	637.61 ± 27.94

*Means t = 799.54, 3.32, 4.42, P values < 0.001 compared with DC tumor vaccine treatment group before treatment, t = 2.83, 3.79, 4.25, P values <0.001 compared with the control group after treatment.
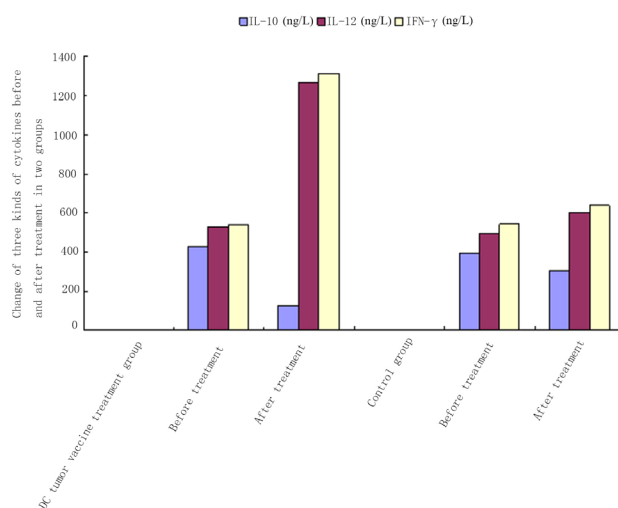

Figure 3. Changes of three kinds of cytokines before and after treatment in the two groups (%, means ± SD).

Adverse reactions and safety evaluation

After being inoculated with DC tumor vaccines, 11 patients experienced fever, with a temperature between 37.4° to 38.3°C; all patients returned to normal in 6 to 24 h without any special treatment. Twelve patients experienced a skin discoloration with a diameter of 1.5-2.6 cm 7 days after the tumor vaccine injection, but all patients were able to tolerate it and the discoloration disappeared on its own without any special treatment after 72 h. During the tumor vaccine

immunization, no patients experienced symptoms such as nausea, vomiting, headache, or arthritis, and we did not observed any autoimmune reactions such as bronchial asthma and anaphylactic shock.

Some patients experienced different degrees of appetite improvement and pain relief, and their quality of life improved dramatically. Routine blood, liver, and kidney function, ultrasound of the abdomen and chest X-rays did not detect any significant abnormalities, and all patients tolerated the treatment very well.

The follow-up situation

The duration of follow-up was 18 months. In the DC tumor vaccine treatment group, five patients of the 80 experienced a local recurrence, six had a distant recurrence, and three had both local and distant recurrences, for an overall rate of recurrence of 17.50%, which was significantly lower than that of the chemical treatment group (48.75%, P < 0.005).

Eleven patients died in the DC tumor vaccine treatment group 11 to 18 months after the operation, for a survival rate of 86.25%, which was significantly higher than that of the chemical treatment group (52.50%, P < 0.005). In the chemical treatment group, 38 patients died 7 to 18 months after the operation, as shown in Table 4. Parts of transfer and recurrence were respectively in areas such as the lung and liver.

Table 4. Comparison of recurrence rate and survival rate in the two groups (%).

Groups	Number of cases	Rate of metastasis and recurrence	Rate of survival
DC tumor vaccine treatment group	80	17.50 (14/80)*	86.25 (11/80)#
Control group	80	48.75 (39/80)	52.50 (38/80)

Compared with control group, *means χ^2 = 10.16, P < 0.005; #means χ^2 = 9.85, P < 0.005.

Preoperative CT of nine cases in the chemical treatment group showed that there was a large block of liver cancer in the left and right hepatic lobes. The mean serum AFP of these patients was 8634.13 ± 11.27 µg/L, IL-10 was 385.36 ± 13.53 ng/L, IL-12 was 473.39 ± 23.47 ng/L and the IFN-γ was 514.57 ± 13.28 ng/L. Chemotherapy was conducted 1 week after eradicative resection of the liver cancer, and the AFP at that time was 897.16 ± 12.45 µg/L, IL-10 was 292.24 ± 13.51 ng/L, IL-12 was 593.26 ± 25.63 ng/L, and the IFN-γ was 611.55 ± 27.43 ng/L. At the sixth month of follow-up, the serum AFP was 897.16 ± 12.45 µg/L, IL-10 was 292.24 ± 13.51 ng/L, IL-12 was 593.26 ± 25.63 ng/L, and IFN-γ was 611.55 ± 27.43 ng/L. CT showed that there were multiple low-density metastases in the right hepatic lobe after the resection of the left hepatic lobe, and there existed multiple low-density metastases in the left hepatic lobe after the resection of the right hepatic lobe.

After three treatments with the DC tumor vaccines the serum of these nine patients was reviewed and AFP was 112.04 ± 11.23 µg/L, IL-10 was 124.13 ± 13.26 ng/L, IL-12 was 867.95 ± 59.83 ng/L, and the IFN-γ was 963.75 ± 27.66 ng/L. CT showed that the low-density foci on the left or right hepatic lobe were narrower than before treatment.

DISCUSSION

Liver resection is the most effective treatment of primary liver cancer, but even after performed radical resection 60-70% of patients relapse within 5 years. At present, systemic chemotherapy for treating hepatocellular carcinoma postoperatively is largely ineffective, and

there are undesirable effects on the immunity of patients. It is critical to explore new treatments to prevent and improve the problems caused by chemical treatments.

As a clinician, designing an effective biological immune therapy for prevention of postoperative recurrence was one of the problems to be solved at present. Early research suggests that DCs are the most powerful APC in the body. They can effectively absorb and process the tumor antigens and express high levels of major histocompatibility antigen complexes such as molecules of class I and II and various stimulus molecules such as CD80 and CD86, which play an important role in the tumor immune response (Mortara et al., 2007; Sun et al., 2010b). Some studies on the use of immunotherapy for treating solid tumors with DCs have been published (Lee et al., 2005). However, little research on enhancing the immune response for prevention of recurrence of postoperative of liver cancer has been performed.

This study induced DCs from the peripheral blood of patients with liver cancer, which were then activated by cytokines such as rhGM-CSF and rhIL-4. They were then induced to mature by TNF-α and sensitized by the cells of the autologous liver tumor. The DC tumor vaccines were injected into patients' bodies to explore their clinical effect on inducing immunity in patients with liver cancer and to assess their ability to prevent the recurrence and metastasis of postoperative liver cancer.

T-cell immunity is the core of the tumor immune response. Subsets of T lymphocytes often appear disordered in the peripheral blood of patients with tumors. CD4+ T cells can secrete cytokines to regulate antitumor immunity, and CD8+ T cells can secrete inhibitory factors that play a role of specific immune inhibition. Therefore, the ratio of CD4+ cells to CD8+ cells can reflect the body's immune function (Ruggieri et al., 2010). CD4+ T cells come in two different types, Th1 and Th2. The Th1 cells mainly mediate cell immune responses, by inducing T cell differentiation to Th1, which play an important role in antitumor immunity. Th2 cells mainly mediate humoral immunity, and induce antibodies to be generated. CD8+ T cells also come in two subtypes, Tc1 and Tc2. Tc1 cells secrete IFN-γ, which has significant cytotoxic activity. Tc2 cells secrete IL-4, which is almost without cytotoxic activity. Tc1 cells are the main effectors of antitumor immune cells, which require IL-2 and IFN-γ secreted by Th1 cells for activation.

After DC tumor vaccines were injected, Tc1, Tc2 and the ratio of CD4+ to CD8+ increased significantly, but there was no effect of DC tumor vaccines to induce the differentiation of Th1 in the body. This study examined the changes of the subtypes of CD3+, CD4+ and CD8+ in peripheral blood. The results showed that after administering the DC tumor vaccines, the number of CD3+, CD4+, and CD8+, and the ratio of CD4+ to CD8+ in peripheral blood were significantly increased. This suggests that this simple and easy method of preparing the DCs, which does not need a specific tumor antigen, can stimulate the body to produce a T lymphocyte immune response. If a more specific antigen had been used it may have induced Th1 to differentiate, which would be beneficial to further stimulate the body's anti-tumor immune response, and significantly improve the T lymphocytes' ability to recognize the tumor cells and induce a specific immune response to the liver cancer. As a result, it would kill the residual cancer cells and inhibit the metastatic recurrence of liver cancer.

DCs, as important APC cells, are becoming more important in the biological therapy of tumors (Lee et al., 2005). However, the number of DC in the tumor is limited, and the tumor can make DC dysfunction, apoptosis and absence of signaling molecule in an immune response, resulting in DC immunocompromised (Mailliard et al., 2000).

This study induced DCs from the mononuclear cells in the peripheral blood of the patients with rhGM-CSF and rhIL-4, and TNF-α was used to induce them to mature, after which lysis of autologous liver cancer cells was used to sensitize them. After injection into the patient, the DCs induce specific T lymphocyte immune cells to kill tumor cells. This method was simple, and it

was not necessary to know the type(s) of tumor antigen, avoiding the difficulties of identification of tumor-specific antigens. This method also stimulated the T lymphocyte immune response to multiple tumor-associated antigens, which can reduce the possibility of tumor escape, as suggested by research conducted by Hoffmann et al. (2000).

At the same time, the diversity of antigen content generated by tumor cracking may lead to the induction of an autoimmune disease. However, our study found that the patients had no serious adverse reactions and had no occurrence of autoimmune disease through clinical observation and 18 months of follow-up. All patients tolerated the treatment well, and had significant improvements in quality of life. There were only 14 cases of recurrence and metastasis in the DC tumor vaccine group, with a recurrence rate of 17.50%, and the survival rate was 86.25% through the eighteenth month of postoperative follow-up. In comparison, there were 39 cases of recurrence and metastasis in the chemical treatment group, and 38 patients died during the period from the seventh to the eighteenth month of postoperative follow-up. The survival rate was only 52.50%, much lower than in the DC vaccine group, and the metastatic recurrence rate was also significantly lower in the DC tumor vaccines group.

There were 12 cases of delayed hypersensitivity among the 80 patients in the DC tumor vaccines group. Delayed hypersensitivity is a kind of immune response mediated by T cells and has been suggested to be a reliability index of the immune response, which showed that DC tumor vaccines had stimulated the immune responses in patients with liver cancer.

Recurrence of liver cancer is thought to be of two kinds. One is the intrahepatic spread of primary tumors. Disseminated lesions continue to grow, and tumor cells can be found upon clinical examination of the liver after resection of the primary tumor. Other is a change on the basis of chronic liver tumor recurrence. In our study, nine cases of recurrence due to intrahepatic metastasis of liver cancer sixth months after surgery in the chemical treatment group were treated with DC tumor vaccines. After three treatment cycles, the recurrent lesions were found to be significantly narrowed, which suggested that DC tumor vaccines are suitable for controlling trace residual cancer cells after curative surgery, which can improve the patient's immune function and prevent recurrences.

The DC tumor vaccine therapy is a good choice for patients with metastatic recurrence. The injection of DC tumor vaccines was only continued for 6 months in this study, which lead to an only limited ability to inhibit recurrence, but there is the possibility of only a limited effect on tumor recurrence, as suggested by the research of Takayama et al. (2000). Our results did not show that the survival rate was significantly improved overall, but the encouraging results have opened up a new approach to liver cancer immunotherapy. The related mechanisms need to be studied more in-depth in future studies.

Immunology indicators that estimate the prognosis of liver cancer include IL-10, IL-12, and IFN-γ. IL-10 was significantly higher in the serum of the patients with liver cancer, but IL-12 and IFN-γ were obviously lower. Single- and multiple-factor analysis has shown that the prognosis of patients with liver cancer with high levels of IL-10 and low levels of IL-12 and IFN-γ is poor (Chau et al., 2000; Wigginton et al., 2001; Parasole et al., 2001; Zhang and Wang, 2006; Airoldi et al., 2009). The results of our study show that treatment with DC tumor vaccines can result in a significant reduction in serum levels of IL-10, and increase IL-12 and IFN-γ, improving the body's anti-tumor immune response and inhibiting the recurrence and metastasis of liver cancer postoperatively.

In conclusion, this study found that treating patients with liver cancer postoperatively with DC tumor vaccines can delay the metastasis and recurrence of liver cancer. However, the curative effect on long-term follow-up is unknown. In addition, overall survival was not found to be obviously improved, for reasons that are not yet clear. We will continue to research this treatment with more cases and a longer period of DC tumor vaccine treatment, or combined with chemotherapy or

targeted therapy in hopes that the effect of the overall treatment will improve the outcomes of patients with liver cancer. The anti-tumor immune liver cancer response, stimulated by DC tumor vaccines, is a promising new biological treatment for liver cancer.

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