Dendritic Cell-Ewing's Sarcoma Cell Hybrids Enhance Antitumor Immunity

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Abstract Given the effective immunotherapy of DC-based vaccine in other cancers, we hypothesized DC-based vaccines would induce effective immune responses against Ewing's sarcoma. To verify this hypothesis and develop the most effective dendritic cell vaccine against Ewing's sarcoma, we evaluated the antitumor efficacy of dendritic cell-Ewing's sarcoma hybrids and dendritic cells pulsed with other antigen-loading methods, including cell lysates and the characteristic EWS-FLI1 gene of Ewing's sarcoma, using an A673 cell line as a model. The hybrids were generated by electrofusion with fusion efficiency and viability determined by flow cytometry and fluorescent microscopy analyses. By interferon- γ secretion assay, the capacity of hybrids to stimulate cytotoxic T-lymphocytes (CTLs) is higher than that of other antigen-loading methods showing stronger tumor antigen-specific CTL cytotoxicity to A673. By in vivo experiment in SCID mice, all dendritic cell-based strategies induced specific immune responses to Ewing's sarcoma after mice-human immune system reconstitution by inoculating human peripheral

blood mononuclear cells into the peritoneal cavity of SCID mice. However, the hybrids most inhibited the subcutaneous tumor growth in SCID mice compared with dendritic cells pulsed with other loading methods. The data suggest A673 cells respond to dendritic cell-based immunotherapy.

Introduction

Ewing's sarcoma is the second most common malignant bone tumor among children and adolescents and constitutes approximately 1% of all childhood tumors. Approximately 95% of Ewing's sarcomas harbor the distinctive chromosome translocation t(11;22)(q24;q12) resulting in the characteristic EWS-FLI1 fusion gene [14], a potential therapeutic target recently explored [33]. Despite the improvements in adjuvant chemotherapy, radiotherapy, and surgery, 30% to 40% of patients with Ewing's sarcoma experience disease recurrence [7, 10] with only a 23% 5-year overall survival rate [4]. Approximately 20% to 30% of children with Ewing's sarcoma present with metastatic disease at diagnosis [25]. Although the overall survival rate of patients without metastasis could reach approximately 60% to 80%, patients with metastasis have an especially poor prognosis. The high percentage of relapse, metastasis, and poor prognosis in patients with Ewing's sarcoma led to renewed interest in alternative immunotherapy.

Dendritic cells (DCs) have been considered the most potent antigen-presenting cells that induce activation and proliferation of naïve CD8⁺ cytotoxic T-lymphocytes (CTLs) and CD4⁺ T helper cells [6, 15, 21]. When the DCs were loaded with tumor antigen, they would present the antigen to T-lymphocyte and activate CD8⁺ T cells in the lymph nodes. Once activated, the CD8⁺ T cells, also known as CTL, would secrete interferon- γ (IFN- γ), leave

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the lymph nodes, and kill the tumor cells by cytolysis and apoptosis [3]. In the last decade, numerous studies have demonstrated DCs loaded with tumor antigen could induce strong *in vitro* and *in vivo* CTL immune responses to the specific tumor [9, 17, 20, 26, 28]. Several antigen-loading methods of DCs have been demonstrated, including tumor antigens, tumor-associated antigen peptides [20], whole protein, nucleic acid (RNA or DNA) [9] tumor cell lysates [17], and DC-tumor cell hybrids [28]. Among these methods, DC-tumor cell hybrids have obtained the most promising antitumor results [26]. Effective immunotherapy of DC-based vaccine has been demonstrated in other tumors such as breast cancer, prostate cancer, and colon cancer [1, 31, 34, 35].

We hypothesized that DC-based vaccines would induce effective *in vitro* and *in vivo* immune responses against Ewing's sarcoma, and the DC-tumor cell hybrids would induce stronger antitumor efficacy than other antigen-loading strategies.

Materials and Methods

To verify this hypothesis, we created DC-A673 hybrids by fusing DCs and Ewing's sarcoma A673 cells and loaded DCs using different antigen-loading strategies. Then we compared the IFN- γ level secreted by CTL stimulated by different DC-based vaccines, examined their CTL cytotoxicity to A673, and explored their *in vivo* antitumor immunity in a nude mice model. We first created DC-A673 hybrids by fusing DCs and Ewing's sarcoma A673 cells through electrofusion, pulsed DCs with the lysates of A673 cells, and modified the DCs with the EWS-FLI1 gene through the adenovirus vector. We then assessed the capacity of the DCs to stimulate CTLs by assessing the IFN- γ secretion from CTLs and the cytotoxicity to the A673 after DC stimulation. Subsequently, we examined the *in vivo* capacity of DCs to stimulate CTL and suppress the tumor growth in nude mice. The mice were inoculated with DCs pulsed by five different antigen-loading strategies to reconstruct the mice immunity. Accordingly, the mice were grouped as follows: (1) DC-A673 hybrids; (2) DCs pulsed with tumor lysates; (3) EWS-FLI1-modified DCs; (4) DCs without antigen loading; and (5) phosphate-buffered saline as a negative control. Each group contained 10 mice. The tumor volumes were measured every 5 days. Comparison of the tumor volumes among different groups was done using a repeated measures test. No samples were excluded from this study.

The human Ewing's tumor cell line A673, which displays characteristic fusion of EWS and FLI1 genes as a consequence of the t(11;22) translocation [18], was obtained from Memorial Sloan-Kettering Cancer Center

and maintained in RPMI medium 1640 supplemented with 10% fetal calf serum (FCS). Recombinant replication-deficient adenovirus encoding the EWS-FLI1 gene was constructed previously in our laboratory by one of the authors (HQ) [23].

Human peripheral blood mononuclear cells (PBMCs) were isolated from healthy volunteer donors by Ficoll-Hypaque density gradient centrifugation suspended in serum-free medium. After 2 hours incubation at 37°C, the nonadherent cells were washed with phosphate-buffered saline. The adherent cells were cultured in RPMI medium 1640 plus 10% human AB serum containing 1000 U/mL granulocyte-macrophage colony-stimulating factor (GM-CSF) and 1000 U/mL interleukin-4 (Human Disease Genomics Center, Peking University). On Day 7, DC maturation was induced by the addition of 1000 U/mL tumor necrosis factor- α . On Day 9, mature DCs were then harvested [5]. After the mature DCs were washed with phosphate-buffered saline and incubated with antibodies, HLA-DR-APC, CD80-PE, CD86-FITC, CD83-APC, and CD14-APC (Becton Dickinson, San Jose, CA) for 15 minutes at 4°C, the cells were analyzed by flow cytometry [3]. HLA-DR, CD80, CD86, and CD83 were the characteristic surface molecules of DCs, and CD14 was the characteristic molecule of monocytes [3] so the flow cytometry assay of these molecular markers could identify the DCs from monocytes.

A673 cells were irradiated with 30 Gy by ^{60}Co before fusion. DCs and irradiated A673 cells were mixed at a 1:1 ratio in a specially designed concentric fusion chamber. Electrofusion was carried out by an ECM 830 (BTX Instruments, Human Disease Genomics Center, Peking University). Fused cells were allowed to rest for 5 minutes [30, 36]. Then, they would either immediately undergo fluorescence activated cell sorting (FACS) analysis for assessing percentage of hybrids or be cultured overnight. After that, cultured hybrids would be used as the vaccine in the animal model burdened with Ewing's sarcoma.

To detect fused cells and quantify fusion efficiency, cells were labeled with membrane dyes in some cases and analyzed by FACS. DCs were stained for 30 minutes in dark with 2.25 μM orange fluorescent CMTMR CellTracker dye (Invitrogen, Beijing, China) and tumor cells were stained with 1.25 μM of green fluorescent CMFDA CellTracker dye (Invitrogen) simultaneously. The stained cells were washed twice and prepared for fusion. After fusion, the cells were assessed by fluorescent microscopy to confirm successful fusion of DC and A673. Fusion efficacy was evaluated by flow cytometry [13, 22].

The A673 cell suspension in phosphate-buffered saline was frozen in liquid nitrogen for 1.5 minutes and then thawed in a 37°C water bath for 4 minutes. The freeze-thaw cycle was repeated three times in rapid succession.

After being cultured with GM-CSF and interleukin-4 for 6 days, monocyte-derived immature DCs were collected and incubated with whole cell lysates of A673 at a ratio of 1:3. After 12 hours of incubation, the pulsed DC maturation was induced by the addition of 1000 U/mL of tumor necrosis factor- α and the nonadherent lysate-pulsed mature DCs were harvested [8].

A total of 3000 μ L of recombinant replication-deficient adenovirus encoding EWS-FLI1 was added (at a MOI of 100) into each 75-cm² flask containing mature DCs. After 1 hour of infection, the adenovirus solution was removed and replaced with fresh culture medium. Twenty-four hours after incubation, the nonadherent cells were collected and used as EWS-FLI1 gene-modified DCs [12, 23].

The CD8⁺ T cells were isolated from the human PBMCs. The DC-A673 hybrids, A673 lysate-loaded DCs, EWS/FLI1-modified DCs, and DCs were mixed with the CD8⁺ T cells at a ratio of 1:10. The mixture was incubated at 37°C for 8 days in RPMI growth medium containing 5 ng/mL interleukin-12 and 20 U/mL interleukin-2. T cell activation was determined by Trypan blue exclusion test. The supernatants were harvested everyday. Interferon- γ release assay was performed by enzyme-linked immunosorbent assay (Genzyme, Cambridge, MA). Each assay was performed in triplicate. To determine whether hybrids stimulated a tumor cell-specific CTL response, the cytotoxicity assay was measured by a ⁵¹Cr-release assay kit (Promega, Beijing, China) using the cultured A673 tumor cells as target cells and stimulated CTLs as effector cells. CTL effector cells and ⁵¹Cr-labeled tumor cells were mixed in a 24-well plate at the various effector/target ratios (E:T; 90:1, 30:1, 10:1). The mixtures were incubated at 37°C in 5% CO₂; after 4 hours, 50 μ L of supernatant was collected and the percentage of killed cells was calculated by the following formula: % release = 100 $\times$ [(cpm experiment – cpm spontaneous release)/(cpm maximum release – cpm spontaneous release)]. The spontaneous release groups lack CTL effector cells, and the maximum release groups contain 2% sodium dodecyl sulfate to activate the maximal ⁵¹Cr-release from the tumor cells.

We obtained 5-week-old NOD/SCID male mice from Peking University Animal Center. The human PBMCs were cultured overnight in RPMI 1640 plus 10% FCS and 20 U/mL interleukin-2. Then the immunity of the SCID mice was reconstructed through intraperitoneal injection with the human PBMC at a dose of 5.0×10^7 cells per mouse. One week later, 2.5×10^6 Ewing's sarcoma A673 cells in 400 μ L phosphate-buffered saline was inoculated subcutaneously into the right flank of the mice, and the DC vaccine that was HLA-matched to the PBMCs was injected into the sole of the mice foot and the root of the mice nails (areas with a high concentration of lymph nodes). Each mouse was given the DC vaccines twice every 3 days. All

the DC vaccines injected in the mice were irradiated with a single dose of 30 Gy to kill residual A673 and ruin the nucleus of DC cells to avoid the heterogametic heritage of the vaccines. The residual A673s would also be killed; the mice that would be inoculated differently from those receiving DC vaccines were grouped as described subsequently. Each group contained 10 mice. The first group was given DC-A673 hybrids. The second group was given DCs pulsed with tumor lysates. The third group was given EWS-FLI1-modified DCs. The fourth group was given DCs without antigen loading. The fifth group was given phosphate-buffered saline as a negative control. Human IgG serum concentrations in the mice blood were monitored every 5 days by quantitative enzyme-linked immunosorbent assay (Genzyme) according to the manufacturer's instructions. Tumor size was measured every 5 days by an orthopaedic oncologist (TS) at the Musculoskeletal Tumor Center, Peking University People's Hospital. Tumor volume was calculated according to the following formula: $V = ab^2/2$ (V, volume of the tumor; a, long axis of the tumor; b, short axis of the tumor).

We determined the difference of cytotoxicity among the five groups by one-way analysis of variance. The differences in the secreted IFN- γ level and the tumor volume among the groups were determined by repeated measures ANOVA owing to multiple sampling times for these variables. The statistical package SPSS was used to analyze the data (SPSS Inc, Chicago, IL).

Results

The phenotype of mature DCs exhibited typical characteristics with negative CD14 and positive CD80, CD83, CD86, and HLA-DR (Fig. 1).

The hybrids were successfully generated by fusion of the DCs and Ewing's sarcoma A673 cells through electroporation. After staining, A673 cells showed a green color (Fig. 2A). Dendritic cells showed a red color (Fig. 2B). DC-A673 hybrids, the fusion cells of DCs and A673, showed a merged color (yellow; Fig. 2C). The fusion efficiency of DCs and A673 was approximately 16.32% (Fig. 2D).

Hybrids enhanced primary T-cell responses. Trypan blue exclusion test showed T cells were activated (data not shown) 6 days after the DC stimulation. After activation, CTLs secreted high levels of IFN- γ . The IFN- γ secretion from the hybrids was the highest when comparing the hybrid group with the lysate-loaded DC group, EWS-FLI1 gene-modified DC group, and dendritic cell control group respectively ($P = 0.00000005$, $P = 0.0000000035$, and $P = 0.0000000007$) (Fig. 3). Secretion from the CTL stimulated by lysate-pulsed DC, EWS-FLI1-modified DC,

Fig. 1 Phenotypic analysis of the mature dendritic cells (DCs) by flow cytometry is shown. Dot plots show typical expression of mature DC: CD14⁺, CD80⁺, CD83⁺, CD86⁺, and HLA-DR⁺. Reproduced

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Fig. 2A–D Characteristics of DC-A673 hybrids produced by fusing dendritic cells (DCs) and Ewing's sarcoma A673 cells through electrofusion are shown. The cells were stained with CMFDA and CMTMR as described in Materials and Methods. Representative pictures were taken under fluorescent confocal microscopy and the fusion efficiency of DCs and A673 through electrofusion was analyzed by flow cytometry. (A) A673 cells were stained with CMFDA (green color). (B) Dendritic cells were stained with CMTMR (red color). (C) DC-A673 hybrids (fusion cells of DCs and A673)

showed a merged color (yellow color, indicated by arrows) as a result of fusion of DCs (stained with CMTMR, red color) and A673 (stained with CMFDA, green color). (D) Fusion efficiency of DCs and A673 was analyzed by flow cytometry after staining with CMFDA and CMTMR. Upper right quadrant of the dot plot represents the fusion cells with dual fluorescence with a fusion efficiency of 16.32%. Reproduced with permission from the Musculoskeletal Tumor Center, People's Hospital, Peking University (*Beijing Da Xue Xue Bao.* 2007;39:403–408).

and the nonmanipulated DC was at a lower level and gradually reduced ($p < 0.05$) in the previously mentioned order.

In the cytotoxicity assay, CTLs stimulated by the hybrids displayed enhanced activity (50.91% specific killing; E:T ratio, 90:1) compared with that from lysate-pulsed DC (25.43% specific killing; E:T ratio, 90:1; $p = 0.025$), EWS-FLI1-modified DC (14.19% specific killing; E:T

ratio, 90:1; $p = 0.014$), and nonmanipulated DCs (13.33% specific killing; E:T ratio, 90:1; $p = 0.01$); and there was no difference detected between EWS-FLI1-modified DC and nonmanipulated DC ($p = 0.789$) (Fig. 4).

In the SCID mice model, human IgG was detectable in the serum of all mice, indicating the reconstitution of the human immune system in the mice was successful (Table 1). Hybrid-vaccinated mice formed smaller tumors

Fig. 3 Interferon- γ (IFN- γ) secretion from cytotoxic T-lymphocytes (CTLs) stimulated by dendritic cells (DCs) loaded with different antigen-loading methods is shown. Column, IFN- γ secretion from CTL (pg/mL); bars, standard error. (H) DC-A673 hybrids. (L) Dendritic cells loaded with cell lysate of A673. (E) Dendritic cells modified by the EWS-FLI1 gene. (D) Dendritic cell control without antigen loading. Reproduced with permission from the Musculoskeletal Tumor Center, People's Hospital, Peking University (*Beijing Da Xue Xue Bao*. 2007;39:403–408).

Fig. 4 Cytotoxicity of cytotoxic T-lymphocyte (CTL) against A673 was analyzed by ^{51}Cr -release assay. The sensitized CTLs by different dendritic cells (DCs) were used as the effector cells and A673 as the target cells. The CTLs and tumor cells were mixed at three effector/target ratios (90:1, 30:1, 10:1). Column, percentage (%) of A673 lysis caused by CTL; bars, standard error. (H) DC-A673 hybrids. (L) Dendritic cells loaded with cell lysate of A673. (E) Dendritic cells modified by the EWS-FLI1 gene. (D) Dendritic cell control without antigen loading. Reproduced with permission from the Musculoskeletal Tumor Center, People's Hospital, Peking University (*Beijing Da Xue Xue Bao*. 2007;39:403–408).

than those vaccinated with lysate-loaded DC, EWS-FLI1 gene-modified DC, dendritic cell control, and phosphate-buffered saline (PBS) control ($P = 0.039$, $P = 0.00003$, $P = 0.0005$, and $P = 0.000000$ respectively) (Fig. 5). We observed no difference in mice tumor volume between the lysate pulsed DC group and the nonmanipulated DC group

or the EWS-FLI1-modified DC group and the nonmanipulated DC group (Fig. 5).

Discussion

Given that effective immunotherapy of DC-based vaccine has been demonstrated in breast cancer, prostate cancer, and colon cancer [1, 31, 34, 35] we hypothesized that DC-based vaccines would induce effective immune responses against Ewing's sarcoma. By creating DC-A673 hybrids and comparing the antitumor efficacies of DCs pulsed with different antigen-loading strategies, we verified this hypothesis and confirmed the optimal antigen-loading strategy, DC-A673 hybrids.

One of the limitations of our study is that the antigens processed and presented by DCs are not known. It is difficult to monitor the immune response in vitro and identify the contribution of one specific antigen to the total immune response, which may affect the comparison of different antigen loading strategy. In our study we only compared the total immune responses of different antigen loading strategy (shown as the IFN- γ secretion and tumor volume). If DC immunotherapy is subsequently used in patients, the antitumor effect of DC vaccine will also be caused by its total immune response. However, the identification of specific antigen that plays a key role in the induction of the immune response is also important, because it can reduce the side effect created by total antigens presented by DCs if used in patients. We are attempting to identify that antigen. Finally, the DC-based vaccine is created in vitro: when used in vivo, it may show little or no effect. During the freeze-thaw cycle to produce tumor lysate, the tumor cells were killed and part of the antigens was denatured and split with their size and spatial conformation changed [19]. As a result of the denatured tumor antigens, part of the antigen loaded on DCs may not be the native antigen in the active tumor cells [19]. Therefore, although we found manipulated DCs could induce specific CTL responses, the effector cells such as CTLs could not induce effective cytotoxicity to the A673 cells or suppress the growth of the tumor inoculated in the mice.

Dendritic cells are the most potent stimulators of primary immune responses ever known, which have been recognized as potential tools for immunotherapy and vaccine strategies. Effective immune responses against tumor are rarely observed among patients as a result of many mechanisms involved, eg, generation of antigen-loss tumor variants, loss of MHC expression, and downregulation of antigen-processing machinery [29]. To augment the host immune response against tumor, DC-based immunotherapy was recently extensively introduced, because the mature DCs with high expression of MHC and costimulatory

Table 1. Serum IgG levels ($\mu\text{g/mL}$) in the serum of SCID mice monitored at 3, 21, 28, 35, 42, and 49 days after immune reconstitution with human PBMCs

DC vaccines and controls	Days after PBMC immune reconstitution in SCID mice					
	3 Days	21 Days	28 Days	35 Days	42 Days	49 Days
PBS	0.79 ± 0.51	1063.83 ± 362.02	2394.73 ± 483.22	1527.25 ± 198.02	1364.28 ± 232.14	1271.13 ± 137.94
DC	0.56 ± 0.48	1315.68 ± 463.93	2954.30 ± 378.23	2416.34 ± 301.19	1684.32 ± 437.00	1525.98 ± 237.23
DC/EWS-FLI1	0.53 ± 0.37	984.36 ± 401.99	1745.23 ± 348.83	2174.34 ± 423.14	2813.91 ± 337.01	1476.74 ± 197.37
DC/lysate	0.64 ± 0.21	1143.01 ± 230.12	2536.22 ± 537.01	2178.12 ± 1382.23	1847.98 ± 243.50	1983.93 ± 273.33
Hybrids	0.64 ± 0.66	1263.83 ± 362.02	1892.44 ± 843.23	2332.98 ± 401.90	2737.01 ± 232.14	2493 ± 327.28

Serum IgG levels ($\mu\text{g/mL}$) are denoted as mean $\pm$ standard deviation; PBMC = peripheral blood mononuclear cell; PBS = phosphate-buffered saline; DC = dendritic cell.

Fig. 5 Antitumor effect of dendritic cells (DCs) loaded with different antigen-loading methods is shown. Each group contained 10 SCID mice. Each mouse was subcutaneously inoculated 2.5×10^6 A673 cells into the right flank the seventh day after the immune reconstruction by injecting the human peripheral blood mononuclear cells at a dose of 5.0×10^7 cells per mouse into the peritoneal cavity. DC vaccines were injected into the sole of mice feet and the root of the mice nail where lymph nodes were enriched. Then DC vaccine inoculation was repeated twice every 3 days. Animals were euthanized on the 25th day after tumor cell inoculation. Tumor growth curves were plotted from the mean tumor volume $\pm$ standard error measured every 3 days from 10 animals in each group. Points, mean tumor volume; bars, standard error. (H) DC-A673 hybrids. (L) Dendritic cells loaded with cell lysate of A673. (E) Dendritic cells modified by the EWS-FLI1 gene. (D) Dendritic cell control without antigen loading. PBS = phosphate-buffered saline. Reproduced with permission from the Musculoskeletal Tumor Center, People's Hospital, Peking University (*Beijing Da Xue Xue Bao.* 2007;39:403–408).

molecules could effectively present the tumor antigen to T cells after in vitro-loaded tumor antigen, thus inducing effective specific immune responses [3, 9, 16, 17, 20]. During the last decade, numerous studies demonstrated the DC-based vaccines are effective in preventing genesis and development of various tumors [1, 31, 34, 35], but few in

Ewing's sarcoma. Using Ewing's sarcoma cell line A673 as a model, we verified the efficacy of DC-based Ewing's sarcoma vaccine. The result would stimulate interest in clinically investigating applying DC-based immunotherapy for Ewing's sarcoma.

Among all four tumor antigen-loading strategies, the DC-A673 hybrid is the most competent. The fusion of DCs and A673s has the advantage that the antigen does not need to be identified [28, 30, 34, 35]. Hybrid DCs can present all the antigens of the entire tumor cell to effector cells, especially the tumor antigens that have not been identified but might be crucial to tumor immunity. Therefore, stronger and specific immune responses against Ewing's sarcoma were induced. However, this strategy has the disadvantage that HLA-matched tumor cells are needed for preparation and it is more difficult to monitor immune responses because the antigens are not known. Moreover, the induced immune responses were complex. Other immunologic cells in SCID mice may also be activated and induced such as NK cells, regulatory T cells, and CD4+ T cells. However, we have not explored their immune activity in this study. Besides this four tumor antigen-loading strategy, there are also other methods that we have not explored here. Further experiments are underway to elucidate them.

DC-A673 hybrids were generated by electrofusion, a newer technique that seems superior to traditional chemofusion [11]. The chemical fusogen polyethylene glycol (PEG), which can cause hygroscopic aggregation and shrinkage of cells, mimics other physical methods to bring cells into contact [24]. The fusion rate of cells increases with the concentration of PEG, but cell survival is substantially affected by combining PEG incubation with either DC alignment or centrifugation. Furthermore, it is toxic for the animal and clinical applications [32]. Therefore, we believe the electrofusion strategy has a potential application for DC-based immunotherapy.

We found EWS-FLI1-modified DCs had no advantage over nonmanipulated DCs. Ewing's sarcoma is characterized by chromosomal translocation $t(11;22)(q24;q12)$,

producing the EWS-FLI1 fusion gene encoding for a chimeric protein, a protein considered the cause of this malignant bone tumor as a transcriptional activator [27]. A673 has the characteristic fusion gene EWS-FLI1. Although we found EWS-FLI1-modified DCs could induce effective in vitro antitumor immune response, the in vivo antitumor immune response was similar between EWS-FLI1-modified DCs and nonmanipulated DCs. A possible explanation is chimeric protein is a DNA-binding and transcriptional activation element mainly located in the nucleus of the tumor cells and is rare on the membrane [2]. The modified DCs could induce specific immune responses because of the ectopic overexpression of the EWS-FLI1 gene in DCs, but the effector cell could not induce an effective immune response because of the lack of the epitope-EWS-FLI1 on the membrane of the tumor cells. Thus, although more IFN- γ was secreted by the CTLs (induced by modified DCs), the cytotoxicity of the CTLs was not superior to the nonmanipulated DCs.

By comparing the immunogenicity of several DC-based vaccines for Ewing's sarcoma, we observed a substantial antitumor effect of a DC-based vaccine on Ewing's sarcoma, and the DC-tumor cell hybrids were the most effective among all the antigen-loading strategies of DCs. Despite limitations of the approach, the DC-tumor cell hybrid vaccine may be an attractive and promising therapeutic strategy for patients with Ewing's sarcoma. The detailed effectiveness and toxic effect of the hybrids need further investigation before clinical use.

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