Electrofusion of syngeneic dendritic cells and tumor generates potent therapeutic vaccine

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Abstract

Antigen presentation by dendritic cells (DCs) has the potential to elicit therapeutic immune responses against malignant tumors. One strategy utilizing DC-tumor fusion hybrids as cancer vaccine is particularly attractive because of polyclonal presentation of a diverse array of unaltered tumor antigens. We have recently developed a large-scale electrofusion technique for generating DC-tumor heterokaryons and demonstrated their superb immunogenicity. Here, employing the weakly immunogenic MCA205 sarcoma, a single vaccination with electrofusion hybrids eradicated tumors established in the lung, skin, and brain. Immunotherapy required intra-lymphoid vaccine delivery and co-administration of adjuvants such as OX-40R antibody. Tumor eradication was immunologically specific and involved the participation of both CD4 and CD8 T cells. Consistent with DC/C213 functionality of MHC-restriction, the use of syngeneic DCs for fusion was an obligatory requirement. Fusion with allogeneic DCs completely lacked therapeutic effects. These findings provide a strong impetus for treating cancer patients with similarly generated DC-tumor hybrids.

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1. Introduction

Using dendritic cells (DCs) as a vehicle to present weak tumor-associated antigens is theoretically attractive in therapeutic vaccine design [1–3]. DC-based cancer immunotherapy has been intensively investigated in pre-clinical animal tumor models and in humans during the past decade. The form of antigens loaded onto DCs ranges from minimal MHC class I-restricted peptides to proteins [4,5]. Loading DCs with a diverse or broad spectrum of tumor antigens would lessen the chance for escape. This has generally been accomplished by transfecting DCs with tumor-derived RNA and DNA, pulsing DCs with necrotic or apoptotic tumor cells, tumor lysates or tumor-derived exosomes [6–8]. In most of these studies, DC-based vaccines have demonstrated their effectiveness prophylactically and in some cases, therapeutically against established tumors when mice were repeatedly immunized. Because of variations in DC progenitor selections and culture systems, individual cell populations may display significant disparities in their lineage, maturation stages as well as their subpopulation functions. Furthermore, tumor models with varying degrees of immunogenicities as well as growth rates were employed in these analyses. As a result, there is no general consensus of optimal approaches for DC immunotherapy. Thus, despite recent advances, DC-based immunotherapy remains a challenge and several parameters need to be optimized in order to maximize their therapeutic efficacy.

To further develop effective DC-based strategies, fusion of DCs with tumor cells is particularly attractive. It offers the theoretical advantage of ensuring a broad and continuous source of unaltered tumor antigens as well as superior antigen presenting functionality of DCs. Several studies have shown that fusion cells were functionally active in stimulating both CD4 and CD8 T cells and eradicating established tumor metastases [9–11].

Abbreviations used: DCs, dendritic cells; H12, a cloned cell line of the MCA205 sarcoma; Adh., adherent; N.Ad., nonadherent; i.s.p., intra-splenic; i.n., intranodal (intra-lymph node).

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However, the traditional fusion method using polyethylene glycol (PEG) is often plagued by widely ranging efficiencies, toxicity, poor reproducibility, and varying susceptibilities among individual tumor cell partners. In addition, the documented effectiveness of employing allogeneic DC fusions defied the very foundation of the principle of MHC restriction [12–14]. Because co-mingling of DCs and tumor cells is a pre-requisite for fusion, the observed reactivities may reflect a heightened immunogenicity due to antigen uptake, costimulation and/or adjuvant activities associated with allogeneic immune responses.

We have recently described an alternative means of generating DC-tumor hybrids by exposing cells to electric fields [15,16]. The success of fusion has unequivocally been verified by a number of analyses including FACS, cytospin, confocal immunofluorescence, and DNA content. Our previous studies demonstrated their therapeutic efficacy against tumors express a surrogate antigen, β-galactosidase, and their superior immunogenicity to peptide- or protein-loaded DCs for stimulating specific IFNγ secretion from both CD4 and CD8 immune T cells. In this study, we extended the analysis of the therapeutic potential of DC-tumor fusion hybrids for the treatment of the unmodified, weakly immunogenic murine sarcoma, MCA205. In addition to pulmonary metastases, tumors established in the skin and brain were also susceptible to the immunotherapy. Furthermore, our study revealed the obligatory requirement of syngeneic DCs for generating therapeutic fusion cells, which is consistent with the MHC restriction of DC’s ability to present antigens to the immune system.

2. Materials and methods

2.1. Mice

Female C57BL/6N (B6, H-2b) and Balb/c (H-2d) mice, 6–8 weeks old, were purchased from the Biologic Testing Branch, Frederick Cancer Research and Development Center, National Cancer Institute (Frederick, MD). The animals were housed in a specific pathogen-free environment and were used for experiments at the age of 10–14 weeks in accordance with National Institutes of Health guidelines.

2.2. Tumors

The MCA205 and MCA207 fibrosarcomas are 3-methylcholanthrene-induced tumors of B6 origin [17]. The tumors have been routinely passed in vivo by serial s.c. transplantation in syngeneic mice and were used within the fifth to the tenth transplantation generation. Single cell suspensions were prepared from solid tumors by digestion with a mixture of 0.1% collagenase, 0.01% DNase, and 2.5 U/ml hyaluronidase (Sigma, St. Louis, MO) for 3–4 h at room temperature. The H12 is a clone of the MCA205 sarcoma, selected for stable growth characteristics and H-2b expression. The H12 clone and the MCA205 sarcoma shared cross-reacting tumor-rejection antigens. H12 cells were maintained in culture at 37°C, 5% CO2 in complete medium (CM) as previously described [18]. Cultured H12 cells were used for electrofusion and MCA205 cells were used to establish tumors in mice for immunotherapy.

2.3. Electrofusion of DCs and H12 tumor cells

DCs were generated from spleens of B6 or Balb/c mice after eight consecutive daily i.p. injections of 5 μg Flt-3 Ligand (a gift from the Immunex, Seattle, WA) as previously described [19]. CD11c cells were enriched by positive selection using MACS CD11c MicroBeads (Miltenyi Biotec, Auburn, CA) following manufacturer’s instructions. Purified cells were cultured for an additional day at 37°C, 5% CO2 in CM containing GM-CSF (10 ng/ml) and IL-4 (10 ng/ml).

For electrofusion, irradiated (5000 cGy) H12 tumor cells were labeled with carboxyfluorescein diacetate succinimidylic dialdehyde (CFSE) (Molecular Probes, Oregon). Technique and procedures for electrofusion have been previously described in this laboratory [15,16]. Briefly, DCs and tumor cells at 2:1 ratio were suspended in fusion medium. Fusion medium consisted of 5% glucose solution containing 0.1 mM Ca(CH3COO)2, 0.5 mM Mg(CH3COO)2, and 0.3% BSA (pH adjusted to 7.2–7.4 with L-histidine). After centrifugation, the pellets were resuspended in the same fusion medium without BSA at a concentration of 15 x 106 cells/ml. Electrofusion was carried out using a custom designed concentric fusion chamber connected to a pulse generator (ECM 2001, BTX Instrument, Genetronics, San Diego, CA). Fusion was accomplished by dielectrophoresis with an ac pulse of 217 V/cm for 10 s followed by a dc pulse of 1228 V/cm for 99 μs. The fusion mixture was then resuspended in CM and incubated overnight at 37°C, 5% CO2. The nonadherent (N.Ad.) and adherent (Adh.) cell populations were harvested, washed, and analyzed for fusion efficiencies by staining with PE-conjugated mAbs against DC markers such as CD80, CD86, and I-A.

2.4. Flow cytometric analysis

PE-conjugated mAbs for direct immunofluorescence staining including Thy1.2 (CD90.2), B220 (CD45R), ICAM-1 (CD54), CD4, CD8, CD11c, CD80, CD86, H-2Kb, H-2Kd, I-Ab, and I-A^d mAbs were purchased from BD PharMingen, San Diego, CA. Analyses of at least 10,000 cells were performed for each sample using the
2.5. Active immunotherapy

Pulmonary metastases were initiated by i.v. injections of mice with \(3 \times 10^5\) tumor cells suspended in 1.0 ml Hanks’ balanced salt solution (HBSS). To establish intracranial tumors, mice were anesthetized with pentobarbital followed by inoculation with \(1 \times 10^5\) tumor cells suspended in 10 \(\mu\)l of HBSS tran cranially as previously described [20]. Subcutaneous tumors were established by inoculating mice in the right flank with \(1 \times 10^6\) tumor cells in 100 \(\mu\)l of HBSS.

For immunotherapy, B6 mice with 3-day established tumors were vaccinated by the intrasplenic (i.sp.) route with fusion cells. After anesthetized, 0.6–1 \(\times 10^6\) Adh. fusion cells were administered in groups of five to ten mice, through a small incision on the left flank. In some experiments, intranodal (i.n.) injections of mice were done following anesthesia as described previously [15,16]. Mice that received fusion cell vaccination were also treated with an i.p. injection of 150 \(\mu\)g OX-40R mAb (a gift from Dr. Andrew Weinberg, Earle A. Chiles Research Institute, Portland, OR). In some experiments, 4-1BB mAb (150 \(\mu\)g, mAb 2A, provided by Dr. Lieping Chen, Department of Immunology, Mayo Clinic, Rochester, MN) was used as an adjuvant [21]. To evaluate the therapeutic efficacy, mice with pulmonary metastases were sacrificed on day 18, and metastatic tumor nodules on the surface of the lung were counted after counterstaining with India ink as previously described [17]. The diameters of s.c. tumors were measured twice weekly with a Vernier caliper, and size was recorded as an average of perpendicular measurements and presented as individual tumor growth curves. For mice with intracranial tumors, survival was monitored as the endpoint.

2.6. Depletion of T-cell subsets in vivo

Ascites containing the rat IgG2b mAb against the murine CD4 (GK1.5, L3T4) or CD8 (2.43, Lyt 1.2) was produced in sublethally irradiated (500 cGy) cyclophosphamide (2 mg) treated DBA/2 mice. Mice were depleted of T-cell subsets by i.v. injections of 0.2 ml of ascites fluid diluted to 1.0 ml with HBSS one day before active immunotherapy. T-cell depletion was \(\geq 80\%\) as confirmed by flow cytometric analyses of spleen cells from treated mice.

2.7. Statistical analysis

The significance of differences in numbers of pulmonary metastases between groups was analyzed by the Wilcoxon-rank sum test. A two-tailed \(p\) value of \(\leq 0.05\) was considered significant.

3. Results

3.1. Characteristics of DC-tumor hybrid cells generated by electrofusion

Murine DCs were generated from spleens of mice injected with Fli-3 Ligand. They displayed a characteristic mature phenotype with high expression of MHC class I and II, costimulatory CD80 and CD86 molecules as well as the adhesion molecule, ICAM-1 (CD54) on their surface (Fig. 1A). By contrast, MCA205/H12 (H12) tumor cells did not express DC-associated molecules, albeit the expression of MHC class I antigens. After electrofusion and overnight incubation, the presence of fusion hybrids in Adh. and N.Ad. cell populations were analyzed separately. Because tumor cells were pre-labeled with CFSE, fusion cells were detected as double positive cells by FACS analyses following staining with PE-conjugated mAbs against molecules expressed on DCs only. The majority of heterokaryons of DCs and tumor cells were in the Adh. cell population (Fig. 1B). This cell population also contained a significant number of tumor cells. The N.Ad. cell population contained mostly non-fused DCs. Routinely, electrofusion generated approximately 40–60\% of heterokaryonic hybrid cells in the Adh. cell population. Of note is that despite the expression of many DC markers, fusion cells showed down-regulation of CD11c. Furthermore, fusion of tumor cells alone generated homologous hybrids without alteration of their phenotype (Fig. 1C).

3.2. Requirements for effective immunotherapy with DC-tumor fusion hybrids

Therapeutic effects of fusion cells were evaluated in mice bearing 3-day established pulmonary MCA205 metastases. A single i.p. vaccination with \(1 \times 10^6\) Adh. fusion cells resulted in a significant reduction of the numbers of metastatic nodules (Fig. 2A). The effective therapy required the co-administration of immune adjuvants. We have previously shown that IL-12 was an efficient adjuvant [15,16]. In the current study, we demonstrated that a single i.p. administration of either OX-40R or 4-1BB mAbs (150 \(\mu\)g) was also effective.

Electrofusion required mixing of viable DCs with tumor cells and prolonged (overnight) incubation. This could result in antigen uptake by DCs and/or enhanced immunogenicity of tumor cells in the presence of costimulation. The fusion process also generated homologous fusion of tumor cells and the immunogenicity of such hybrids as well as that of tumor cells exposed to electric fields were largely unknown. This prompted us to examine the therapeutic effects of both Adh. and N.Ad. fractions of fusion as well as fusion generated from tumor cells alone. Treatment of pulmonary MCA205 metastases with the Adh. fraction of fusion...
cells plus OX-40R mAb significantly reduced the numbers of metastases (Fig. 2B). Neither vaccination with the N.Ad. cells nor Adh. tumor-tumor fusion cells were therapeutically effective, indicating that only heterokaryons of DCs and tumor cells were capable of inducing a therapeutic immune response.

The delivery of fusion hybrids by i.sp. injections was found effective in generating therapeutic responses. We therefore examined the effects of different vaccination routes on the therapeutic efficacy of DC-tumor fusion cells. As depicted in Fig. 2C, successful therapy required the administration of vaccine directly into lymphoid organs such as the spleen and lymph nodes, whereas s.c. and i.p. routes of immunization were ineffective. We also attempted to treat mice by the i.v. route of vaccination. However, because of the large size of fusion cells, it was not feasible to evaluate due to the induction of pulmonary embolism and death of the recipient mice.

The specificity of the DC-tumor fusion hybrid-induced therapeutic response was demonstrated by using the antigenically distinct MCA207 sarcoma [17]. Despite eradication of MCA205 metastases by immunization with DC-H12 fusion hybrids, metastases derived from the MCA207 sarcoma were refractory to the identical treatment (Fig. 2D). Thus, DC-tumor fusion hybrids stimulated immune responses only to antigens of the tumor that formed fusion hybrids.

3.3. Syngeneic vs. allogeneic DCs for electrofusion

T cells recognize antigens presented by antigen-presenting cells (APCs) only in the context of, and restricted by MHC molecules. As a general rule, MHC mismatched APCs will not stimulate T cells. However, allogeneic DCs may enhance immune response by inducing host responses to allo-antigens at the immunization site, thus providing an enriched cytokine milieu as well as generation of helper T cells. Therefore, efforts were made to test the immunologic reactivity of fusion cells generated from allogeneic DCs. DCs of both B6 (H-2\(^{b}\)) and Balb/c (H-2\(^{d}\)) mice displayed similar phenotypes and were equally susceptible to electrofusion with H12 tumor cells (Fig. 3A). Mice with 3-day established pulmonary MCA205 metastases were treated by either syngeneic or allogeneic DC-tumor fusion hybrids. Enumeration of metastatic nodules on day 18 of tumor growth clearly demonstrated that effective immunotherapy could only be induced by vaccination with syngeneic DC-tumor fusion cells (Fig. 3B).
expression of both H-2b and H-2d molecules on allogeneic DC-tumor fusion cells (see Fig. 3A), they completely failed to stimulate a therapeutic immune response. This observation suggested a critical role of CD4 T-cell responses for effective immunotherapy. To confirm this, we analyzed the significance of host T-cell subsets in the therapeutic immune response. Tumor-bearing mice were depleted of CD4 or CD8 T cells by the administration of CD4 (GK1.5) or CD8 (2.43) mAbs i.v. 1 day prior to vaccination. Depletion of corresponding T-cell subsets was confirmed by FACS analyses of spleen cells. Enumeration of pulmonary metastases revealed a complete eradication of tumor in T-cell intact animals. By contrast, depletion of either CD4 or CD8 T cells in vivo abrogated the antitumor effects of vaccination (Fig. 4). Thus, both MHC class I- and II-restricted CD8 and CD4 immune responses were necessary and associated with therapeutic effects of fusion cell immunotherapy.

### 3.4. Immunotherapy of tumors established at different visceral organs

Analyses of therapeutic effects of fusion cell vaccine in preceding experiments were performed in a model system where mice with pulmonary metastases were treated. However, immunologic reactivities may vary against tumors established at different anatomic sites [20,22,23]. Thus, the MCA205 tumor established in the skin, as well as in the brain, were used as additional models to test the efficacy of DC-tumor fusion immunotherapy. Mice with 3-day established s.c. tumors were vaccinated i.s.p. with 1 × 10^6 DC-H12 fusion hybrids plus one i.p. administration of OX-40R mAb (150 μg). The treatment resulted in retardation of tumor growth and 6 of 10 treated animals were apparently cured of the s.c. tumor (Fig. 5). Similarly, the same treatment regimen for mice bearing 3-day established intracranial tumors resulted in prolongation of survival and 3 of 6 treated mice were cured (Fig. 6A). Again, effective immunotherapy required administration of both fusion cells and adjuvant. Treatment with OX-40R mAb alone or in combination with either N.Ad. fusion cells or tumor–tumor fusion cells was not therapeutically effective. The three mice cured of intracranial tumor were challenged with MCA205 tumor cells intracranially 60 days after initial tumor inoculation. As shown in Fig. 6B, all cured mice rejected a second tumor challenge, whereas all control normal animals died of intracranial tumors within 20 days. Therefore, DC-tumor fusion cell therapy resulted in the induction of a long-lasting immunologic memory.
4. Discussion

Membrane fusion is an essential step in a variety of cellular processes such as exocytosis, endocytosis, fertilization, mitosis, and myogenesis. Experimentally induced cell fusion by Sendai virus, PEG and other chemical agents has provided a useful model for elucidating the mechanism underlying the fusion processes [24–26]. Somatic cell fusion can also be accomplished by exposing cells to electric pulses [27–32]. Electrofusion does not involve fusogens and the process is synchronous and instantaneous. The fundamental step in electrofusion is reversible membrane breakdown. When short-duration direct current (dc) electric impulses applied across cell membrane exceed a critical threshold, that membrane will become transiently but highly permeable through the formation of micropores. If two cells are touching each other during the process of membrane breakdown, adjacent pores may form channels and lead to the formation of a new spherical hybrid cell. To bring cells in close contact, we chose the application of inhomogeneous alternate current (ac) electric field to induce cell dipole interactions leading to alignment or “pearl-chain” formation of cells. This process called “dielectrophoresis” can be observed under a microscope, thus allowing the investigator to adjust fusion parameters for optimal results.

In immunology, electrofusion has been used for production of mAb-producing hybridomas [33]. However, large-scale fusion for generating clinically useful reagents has been difficult due to the requirement of engineering high-power pulse generators as well as designing suitable large fusion chambers. We have previously described an electrofusion technology with which a large number \(( \geq 200 \times 10^6)\) of cells could be processed at one time without compromising fusion efficiencies and reproducibility [15,16]. Because of the high fusion rate, no additional isolation procedure was needed for their use in immunotherapy experiments.

With the success of electrofusion, the current study demonstrated the therapeutic efficacy of DC-tumor fusion hybrids for the treatment of the chemically induced tumor, MCA205 that expressed naturally occurring tumor-specific transplantation antigens. The ability of DC-tumor fusion cells to stimulate both host CD4 and CD8 T cells and responses of both T cell subsets were essential for mediating tumor regression. This is consistent with the phenotypic characteristics of fusion hybrids and confirmed that DC-tumor heterokaryons indeed processed the immunological components of both DCs and tumor cells. Although the current study presented mostly in vivo therapy experiments, we found that there was a dramatic hypertrophy of the spleen 7 days after vaccination. The splenic T cells of both CD4 and CD8 subsets spontaneously secreted a large amount of IFNγ when cultured in the absence of additional...
stimulation (data not shown). The significance of this observation and their specificity, kinetics and phenotype expression awaits further analyses.

In the literature, most studies of DC-based approaches have utilized both syngeneic and autologous DCs to define their immunogenicity. However, a few reports suggest that fusion of allogeneic DCs with tumor cells was effective for CTL generation and in vivo therapy of established tumors [12–14]. In our study, effective hybrids could only be generated from syngeneic DCs. Phenotype analysis indicated that allogeneic DC-tumor fusion hybrids expressed both H-2b (from the tumor) and H-2d (from Balb/c DCs) but lacked detectable I-Ab molecules. Despite the presence of matched MHC class I molecules, allogeneic fusion cells failed to stimulate a therapeutic response suggesting that the ability to induce a CD8 T-cell response alone was not sufficient for tumor regression. It is also possible that the CD8 T-cell response might not occur in the absence of a CD4 T-cell helper response. This conclusion is supported by our experimental results indicating that prior depletion of either CD4 or CD8 host T cells abrogated the therapeutic effects of DC-tumor fusion cells. Therefore, the documented antitumor immune responses, when allogeneic DCs were used, must be interpreted as a reflection of mechanisms other than a direct antigen presentation. It is likely that heightened immunogenicity is the result of adjuvant activity provided by allogeneic DCs. In some studies, immunogenicity of tumor cells could be augmented by the mere presence of DCs at the immunization site or at the tumor site [34,35].

In previous studies [15,16], we reported the adjuvant activity of IL-12. Because of its multi-functional reactivities involving a variety of immune cells and pathway, the mechanism of IL-12 in the context of DC-tumor fusion immunotherapy is not clear. However, the present results indicate that either OX-40R or 4-1BB mAb was also effective. It is particularly interesting that OX-40R is expressed mostly on recently activated CD4 T cells, suggesting a mechanism of sustaining CD4 T-cell boost for its adjuvant activity [36]. However, it remains to be elucidated if multiple mechanisms can operate independently to support the development of a systemic antitumor immune response.
It has been known for some time that tumors established in different visceral organs may display different susceptibilities to immunotherapy [20,22,23]. For example, exogenous IL-2 administration enhanced the therapeutic efficacy of T-cell adoptive immunotherapy against tumors established in the lung. However, similar IL-2 treatment suppressed the function of transferred T cells for the treatment of intracranial tumors [37]. It is intriguing to observe therapeutic effects against intracranial tumors because the central nervous system is generally considered an immunologically privileged site as a result of the lack of lymphatic drainage and the nature of the blood–brain barrier (BBB) in which tight junctions between cerebral vascular endothelial cells form a physical barrier to the passage of chemicals, Abs and cells [38]. However, our previous studies clearly demonstrated that appropriately activated T cells could traffic to the brain tumor site and mediate its regression [20,39]. Thus, our hypothesis is that vaccination with fusion hybrids stimulated a systemic antitumor immune response and the resulting activated immune T cells are capable of gaining access and crossing the BBB to interact and initiate tumor regression in the brain.

The ability of DCs to migrate to the draining lymph node is critical to their function as APCs. In the mouse, only small numbers (<1%) of the s.c. injected DCs are formed in the draining lymph node [40–42]. To avoid the need for DC migration, direct injection of peptide-pulsed DCs into lymph nodes has demonstrated its efficacy for vaccine administration [43]. We found that the most effective means to induce antitumor immunity was direct delivery of vaccine into lymphoid organs such as the spleen. Under such conditions, a single immunization resulted in tumor regression, presumably due to the induction of a primary immune response. For the treatment of advanced disease (e.g. established for 10 days), it may be necessary to administer the vaccine repeatedly. It would be interesting to investigate whether booster immunization can be effective by other vaccination routes such as s.c. because secondary immune responses may not need lymph organ involvement.

In electrofusion, irradiated viable tumor cells are the source of tumor antigens. Tumor cells also contain a whole array of normal tissue antigens and this might impose limitations on their use. Fusion cell vaccination may induce autoreactive T cells with destruction of tissues expressing relevant antigens [44]. This scenario seems unlikely as revealed by a recent study [45] showing that DCs that phagocytosed dying cells could initiate a transient systemic autoimmunity in normal animals. However, clinical and histological disease was evident only in recipients genetically predisposed to developing autoimmune disease. In another study [46], immunity induced against TRP-2 protected mice from a lethal s.c. B16 melanoma challenge while melanocyte destruction in vivo was only sporadic and primarily restricted to minor depigmentation at the immunization site.

Taken together, our data demonstrate an unparalleled therapeutic activity of DC-tumor fusion hybrids for active immunotherapy of malignant tumors. Electrofusion, compared with other fusion methodologies, allow the processing of a large number of cells with high fusion efficiencies and reproducibility. In principle, most mammalian cells are subjected to the effects of electric fields. Our own experience with human cells has demonstrated the feasibility of electrofusion for generating heterokaryons. Most notably, fusion hybrids generated from PBMC derived DCs and some melanoma cell lines were capable of presenting tumor-associated antigens such as gp100, MART-1, tyrosinase and TRP-2 to defined CD4 and CD8 T cells in an MHC-restricted manner [47]. Clinically, we are analyzing the immune response as well as therapeutic efficacy in patients with metastatic melanoma undergoing DC-tumor fusion cell vaccination.

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References


