REVIEW

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Dendritic/tumor fusion cell-based vaccination against cancer

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Abstract

A promising area of investigation is the use of cancer vaccines to eliminate residual tumor cells. Dendritic cells (DCs) are potent professional antigen-presenting cells able to induce primary immune responses. DCs capture and process antigens into peptides and present them to T cells and B cells through MHC class I and II molecules. An alternative approach to the induction of antitumor immunity is the use of fusions of DCs and tumor cells. In this approach, a broad spectrum of tumor--associated antigens, including those known and unidentified, are processed endogenously and presented by MHC class I and II pathways in the context of costimulatory signals. In animal studies, vaccination with DC/tumor fusion cells results in the elimination of established lung metastasis. Preclinical human studies have demonstrated that DC/tumor fusion cells induce antigen-specific polyclonal cytotoxic T-lymphocyte responses against autologous tumor *in vitro.* In clinical studies, vaccination of cancer patients with autologous DC/tumor fusion cells is associated with immunological and clinical responses in a subset of patients. Future studies should be investigated to improve the immunogenicity of DC/tumor fusion cell preparations. This review provides a general overview of the DC/tumor fusion cell-based vaccine and summarizes some of the recent advances in this field.

Key words: dendritic cells, fusion cell vaccine, tumor immunity, antigen-specific CTL.

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DENDRITIC CELL-BASED VACCINE

The ability of tumors to escape immune responses has been a major obstacle to the development of effective tumor immunotherapy. We are now gaining a clear understanding of the cellular events leading to effective T cell-mediated antitumor immunity. Although several encouraging clinical trials have been reported in recent years, pilot studies have clearly demonstrated that antigen-pulsed dendritic cells (DCs) can generate tumor--associated antigen (TAA)-specific immune responses in humans, some of which are associated with clinical responses [2, 21]. DCs are specialized antigen-presenting cells (APCs) and attractive vectors for cancer immunotherapy [3, 6, 50, 51]. DCs are efficient stimulators of both B and T cells. B cells can directly recognize native antigens through their receptors. However, T cells need the TAAs to be processed and presented to them by APCs. DCs derive their potency from the prominent expression of major histocompatibility complex (MHC) class I and II, costimulatory, and adhesion molecules that provide secondary signals for the activation of naive CD4 and CD8 T cells [3, 6, 50, 51]. The evidence for their ability to act as natural adjuvants in the induction of antitumor-specific cytotoxic T lymphocytes (CTLs) in murine and human models is now overwhelming. Both immature and mature DCs are capable of processing antigens into peptides and presenting them through MHC class I and II molecules [31, 33]. There are two different pathways for TAA presentation by DCs [30]. Endogenously synthesized proteins, such as those in viral infections and certain exogenous antigens, are processed and presented through the MHC class I--restricted pathway to CD8 T cells [18, 24, 49]. In con-

trast, exogenous antigens are processed and displayed in association with MHC class II molecules and recognized by CD4 T cells [50, 52]. Thus, DCs are capable of stimulating both CD4 and CD8 T cells and inducing potent CTLs that can directly kill target cells [4]. It is known now that mature DCs are significantly better at CTL induction due to higher expression of MHC and costimulatory molecules, while presentation of TAAs by immature DCs, in the absence of proper costimulation, may lead to tolerance induction [18]. After TAA uptake and inflammatory stimulation, immature DCs in peripheral tissues undergo a maturation process characterized by the up-regulation of costimulatory molecules, chemokine receptors such as CCR7, and the secretion of cytokines such as interleukin (IL)-12 [3, 10, 50]. During this process, mature DCs migrate to the regional lymph nodes, where they act as effective inducers for primary responses of antigen-specific naive T cells [3, 6, 50].

The use of DCs as a booster of antitumor responses has been considered a promising strategy for cancer vaccine [50]. Several strategies to deliver tumor antigens to DCs have been developed to generate a potent CTL response against tumor cells in murine and human systems. DCs have been pulsed with synthetic peptide derived from the known tumor antigens, tumor cell lysates, apoptotic tumor cells, and tumor RNA [37, 43, 44, 47]. Although the production of cancer vaccines for individual patients has currently been addressed in clinical trials, a major drawback of this strategy comes from the limited number of known tumor peptides available in many HLA contexts. Furthermore, although DCs pulsed with antigen-specific peptides have been used in clinical trials for patients with cancer, the results show that clinical responses have been found in a small number of patients [41, 56].

DENDRITIC/TUMOR FUSION CELL-BASED VACCINE

Animal studies

DC/tumor fusion cells. An alternative approach to the induction of antitumor immunity is the use of fused tumor cells and DCs. This novel approach for cancer immune therapy was developed by Gong et al. [12]. In the DC/tumor cell fusion approach, the entire repertoire of TAAs, including those known and those yet unidentified, are processed endogenously and presented by MHC class I and II pathways in the context of costimulatory signals (Fig. 1) [13, 16, 33]. In addition, since the DC/tumor fusion approach delivers not only the TAA epitopes, but also the genes encoding the TAAs, they can continue to produce TAAs for several days after fusion [31]. The DC/tumor fusion cells can be generated by a chemical membrane destabilizing agent known as polyethylene glycol (PEG) or by electrical pulse.

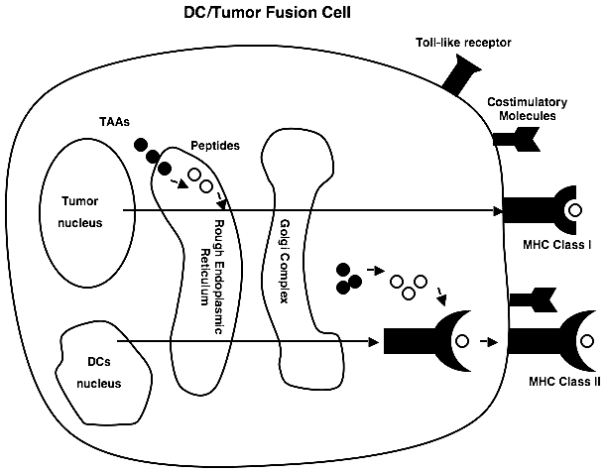

Fig. 1. A model of antigen processing and presentation by DC/tumor fusion cell. Tumor associated antigens can be presented in MHC class I and II pathways in the context of costimulatory molecules.

MUC1 transgenic mice model. In animal studies we have used MUC1-transgenic mice as a preclinical model. MUC1, a carcinoma-associated antigen, is a high-molecular-weight glycoprotein overexpressed in human breast, pancreatic, colon, and other carcinomas [45, 46]. The MUC1-transgenic (MUC1.Tg) mouse model that expresses human MUC1 is unresponsive to MUC1 Ag [46]. Antitumor immunity against MUC1-positive tumors can be augmented by fusions of DCs and MUC1-positive carcinoma cells [13] and this augmented immunity rejects established MUC1-expressing tumor metastases [13, 37, 53]. In contrast, vaccination of DCs transfected with MUC1 RNA could not reverse tolerance to MUC1 [34]. After s. c. injection of the DC/tumor fusion cells, the fusion cells are capable of migrating to draining lymph nodes and are closely associated with T cells in a pattern comparable with that of unfused DCs. Immunization of MUC1-transgenic mice with the DC/tumor fusion cells results in proliferation of T cells and induces MUC1-specific CD8 CTL. Moreover, CD4 T cells activated by DC/tumor fusion cells are multifunctional effectors that produce IL-2, interferon (IFN)-γ, IL-4, and IL-10 [37]. Thus, DC/tumor fusion cells can stimulate both CD4 and CD8 T cells and display full antitumor immunity even in MUC1-transgenic mice. This concept has been confirmed in animal model experiments for many kinds of cancer, including esophageal cancer [17], hepatocellular carcinoma [23, 27], colon cancer [13, 26] lung cancer [48], glioma [1], melanoma [57], and myeloma [14].

Spontaneous mammary carcinoma model. Although the transplantable tumor mice models have been the primary screening tools for cancer vaccine development, they do not fit this criterion since the tumor in these models grows very quickly, without the multiple stages of cancer development found in human cancers. Genetically modified mice with spontaneous develop-

ment of cancer provide a powerful tool to study the efficacy of tumor vaccines, since they mimic cancer development in humans. Vaccination of MMT mice that develop spontaneous mammary carcinoma with DC/tumor fusion cells results in the induction of polyclonal CTL activity against spontaneous mammary carcinoma cells and renders about 60% of the mice disease-free [7, 58]. In other mice models that develop spontaneous hepatocellular carcinoma or gastrointestinal cancer, DC/tumor fusion cells are also capable of inducing antitumor immunity [26, 27]. Importantly, prophylactic vaccination with DC/tumor fusion cells confers sufficient antitumor immunity to counter the tumorigenesis of potent oncogenic products.

Antigen presentation in DC/tumor fusion cells. Effective TAA processing and presentation in DC/tumor fusion cells are crucial to antitumor immunity. To investigate the role of MHC class I-restricted or class II-restricted TAA presentation and the activation of CD4 and CD8 T cells by DC/tumor fusion cells, we had created various types of DC/tumor fusion cells with intact or deficient expressions of MHC class I or II molecules by using several kinds of DCs and tumor fusion partners. The DC/tumor fusion cells had been used in the prevention and treatment of tumors in MUC1-transgenic mice. We observed differential impairment of antitumor immunity induced by fusions of DCs from MHC class I and/or II knockout mice. Immunization with MHC class II-deficient DC/tumor fusion cells abolishes the IFN-γ production of CD4 and CD8 T cells and the induction of CTL, and severely impairs antitumor immunity [54]. The presentation of TAAs on MHC class II is important for the activation of CD4 T cells and the induction of efficient antitumor immunity [54].

The potency of DC/tumor fusion cell vaccination. The potency of DC/tumor fusion cell vaccination has been explored. Several studies of DC/tumor fusion cell vaccines in murine models have reported that the combination of DC/tumor fusion cells with IL-12 [14, 26] or oligodeoxynucleotides containing the CpG motif (CpG ODNs) [19] enhances the antitumor effect more effectively than DC/tumor fusion cell preparations alone. Moreover, IL-12 and IL-18 [25], granulocyte-macrophage colony-stimulating factor (GM-CSF) [5], or IL-4 [40] gene-modified fusion cells can also induce enhanced antitumor immunity. These results suggest the importance of adjuvant to enhance antitumor immunity in the use of DC/tumor fusion cells as cancer vaccines. We have also demonstrated that when combined with IL-12, DC/tumor fusion cell vaccine is effective in the long-term eradication of multiple myeloma [14].

Activation of DC/tumor fusion cells. In peripheral lymphoid organs, immature DCs are incapable of eliciting CTL responses and have been reported to induce tolerance. After antigen uptake and inflammatory stimulation, immature DCs in peripheral tissues undergo a maturation process. Mature DCs are significantly better at CTL induction due to the higher expression of MHC and costimulatory molecules. Interestingly, fusions of tumor cells and immature DCs resulted in the presentation of TAAs and up-regulation of costimulatory molecules such as CD80 and CD86 molecules in a mice study (our unpublished data). However, fusions of CpG ODN-stimulated DCs and tumor cells can induce enhanced CTL, as compared with conventionally prepared DC/tumor fusion cells from immature DCs [8]. DCs fused after maturation may have potential applicability in the field of antitumor immunotherapy.

Selection of DC/tumor fusion cells. There is no standardized method for the selection of DC/tumor fusion cells. Some studies have reported enriching DC/tumor fusion cells by FACS cell sorting and a method to transfect DCs with Tyr-green fluorescent protein reporter virus [20]. The disadvantage of these methods is their complexity and possibility of the insertion of viral genes into the host genome. The other hand, short-time culture of the DC/tumor fusion products can also promote DC/tumor fusion efficiency and reduce cell aggregates [31, 36]. Therefore, it is not necessary to enrich DC/tumor fusion cell preparations using special methods.

Preclinical model

Characterization of human DCs and tumor cells. In preclinical human studies with leukemia [15], breast [11, 36, 38], ovarian [16, 35, 36], and colorectal cancer [31, 32, 33], we had successfully generated DC/tumor fusion cell preparations and demonstrated the induction of CTL responses against autologous tumor cells. DCs from patients with cancer can be generated in GM-CSF and IL-4 medium for 6-7 days and successfully fused to freshly isolated tumor cells by 50% PEG solution. DCs display a characteristic phenotype with expression of MHC class I and II and costimulatory molecules (CD80 and CD86), but not TAAs (CEA and/or MUC1). By contrast, tumor cells express high levels of TAAs (CEA and/or MUC1), MHC class I, but not MHC class II and CD80 and CD86 molecules. Fusion of autologous tumor cells to DCs results in the formation of heterokaryons that express the TAAs and DC-derived costimulatory and adhesion molecules. After fusion, the cytoplasm of the two cells is integrated, whereas their nuclei remain separate entities [36]. In DC/tumor fusion cells, we have demonstrated using electron microscopy the integration of the cytoplasm from both the DCs and the tumor cells and the colocalization of TAAs with MHC class I and II molecules [36]. Such a structure makes it possible to maintain the functions of both original cell types, at least in part, including the synthesis of antigens and costimulatory molecules. The integration of the cytoplasm also makes it possible for TAAs and DC-derived MHC class II and costimulatory molecules, presumably synthesized at separate sites of DC/tumor fusion cells, to converge and complex with each other [36].

Activation of CD4 and CD8 T cells by DC/tumor fusion cells. DC/tumor fusion cells are functional in stimulating the proliferation of CD4 and CD8 T cells.

High levels of IFN-γ production and, to a lesser extent, IL-10 production are detected in both CD4 and CD8 T cells stimulated by DC/tumor fusion cells [33]. Therefore, DC/tumor fusion cells have characteristics to polarize the T cells to a Th1-dominant state, which is critical in suppressing tumors. In addition, IFN-γ production by CD4 and CD8 T cells is blocked by anti-MHC class I and/or class II monoclonal antibody, suggesting antigen presentation by DC/tumor fusion cells through MHC class I and class II pathways [33]. There is increased evidence that CD4 T cells play a critical role in antitumor immunity; thus it will be desirable to use both CD4 and CD8 T cells in adoptive immunotherapy [42, 54, 55]. It is significant that both CD4 and CD8 T cells can be stimulated by DC/tumor fusion cells.

CTL induction by DC/tumor fusion cells. Coculture of DC/tumor fusion cells with patient-derived T cells results in the induction of CTLs, which can show lysis of autologous tumor cells by an MHC class I-restricted mechanism. Although tumor-infiltrating lymphocytes have been cultured only in the presence of high doses of human IL-2 (1000 U/ml), relatively lower doses of IL-2 (10 U/ml) can be used for the culture of T cells stimulated by DC/tumor fusion cells, suggesting that the stimulated T cells may rely less on IL-2 [31]. In adoptive immunotherapy, systemic administration of a high dose of IL-2 may not be necessary for proliferating and maintaining the transferred T cell stimulated by DC/tumor fusion cells *in vitro*, thus eliminating the toxicity by high doses of IL-2. We have demonstrated that administration of T cells stimulated by DC/tumor fusion cells can regress 7-day-old established human tumors in SCID mice and render mice disease-free up to 90 days [38]. Interestingly, DC/tumor fusion cells can be efficiently frozen without loss of either antigen presentation or T cell stimulatory capacity inducing CTL responses against autologous tumor cells [31]. The cryopreserved DC/tumor fusion cells have potential applicability in the field of antitumor immunotherapy and provide a platform for adoptive immunotherapy in the clinical setting.

Allogeneic DC/autologous tumor fusion cells. An autologous DC/autologous tumor fusion cell can present TAAs by tumor or DC-derived MHC class I molecules. Moreover, the fusion cells can also present TAAs by DC-derived MHC class II molecules and thereby stimulate antigen-specific CD4 cells [9, 39]. By contrast, presentation of tumor-derived antigens by allogeneic DC/autologous tumor fusion cells is dependent on tumor-derived MHC class I molecules. Allogeneic DC/autologous tumor fusion cells also stimulate alloreactive T cells to release cytokines, which contribute to enhance and/or maintain TAA-specific CTL responses [16, 53]. When T-cell clones responsive to tumor peptides become anergic, the strong allogeneic stimulation is likely to reverse this state [9]. However, fusions of autologous DCs and autologous tumor cells may be more effective in targeting tumor cells because they can stimulate antigen-specific CD4 cells [9, 39].

Autologous DC/allogeneic tumor fusion cells. In the clinical setting of patients with cancer, a major difficulty in producing a DC/tumor fusion vaccine is the preparation of sufficient amounts of autologous tumor cells. Especially a specimen of tumor from the primary lesion may not provide sufficient numbers of viable tumor cells due to the length of culture time and potential contamination by bacteria and fungus. Fusions of DCs from a healthy donor and an allogeneic tumor cell line can induce CTL responses against the tumor cells used for fusion [38]. In addition, administration of T cells stimulated by DC/allogeneic breast cancer fusion cells regresses seven-day-old established tumors and renders SCID mice disease-free up to 90 days [38]. Thus, the tumor cell line can be used as a fusion partner in the construction of a DC/tumor fusion vaccine. More importantly, autologous DCs fused with an allogeneic colorectal carcinoma cell line can prime autologous T cells to differentiate into antigen-specific CTLs able to kill autologous colorectal carcinoma cells [32]. This strategy has numerous advantages: a) the allogeneic tumor cell line is well characterized as a TAA source; b) the allogeneic tumor cell line, which has shared TAAs, can grow well *in vitro*; thus, there is no limiting factor for the preparation of tumor cells; c) it is not necessary to determine HLA typing of patients and allogeneic tumor cells as a partner in fusion cells because autologous DCs can process and present multiple TAAs from allogeneic tumor cells in the context of MHC classes I and II. Therefore, autologous DC/allogeneic tumor fusion cells hold promise since they can be used as a vaccine for active immunotherapy and as stimulators to activate and expand T cells for adoptive immunotherapy.

Mature DC/tumor fusion cells. Both immature and mature DCs are capable of processing and presenting MHC-peptide complexes to T cells. However, mature DCs are significantly better at CTL induction due to higher expression of HLA and costimulatory molecules, while the presentation of antigens by immature DCs, in the absence of proper costimulation, may lead to tolerance induction. Mature DCs have an enhanced capacity to stimulate autologous T cells as a result of up-regulated MHC and costimulatory molecules. Maturation of DCs can be influenced by lipopolysaccharide, prostaglandin E2 (PGE2), CD40 ligand, and the reported mixture of cytokines (consisting of TNF-α, IL-1β, IL-6, and PGE2). OK-432, a penicillin-inactivated and lyophilized preparation of a low-virulence strain (Su) of *Streptococcus pyogenes* (group A), is one of the biological response modifiers and a good manufacturing practice grade agent. Recently it has been demonstrated that OK-432 modulates DC maturation through Toll-like receptor 4 to enhance TAA-specific CTL responses. We have created hybrid cells by fusing OK-432-stimulated DCs and autologous colorectal carcinoma cells (OK-FCs) [31]. OK-FCs coexpress the CEA and MUC1 and significantly higher levels of CD86, CD83, and IL-12 than those obtained by fusions of immature DCs and autologous colorectal carcinoma cells (Imm-FCs). Interestingly, OK-FCs are more efficient in stimulating

CD4 and CD8 T cells capable of high levels of IFN-γ production and cytolysis of autologous tumor targets. Moreover, OK-FCs are more effective inducers of CTL activation compared with Imm-FCs on a per fusion cell basis. OK-432 can promote DC/tumor fusion efficiency and the induction of TAA-specific CTLs by the fusion cells [31]. Therefore, OK-FCs may have potential applicability in the field of antitumor immunotherapy and provide a platform for adoptive immunotherapy in the clinical setting.

Clinical study

We had conducted a phase I/II clinical study for the safety profile of vaccination with DC/tumor fusion cells and rhIL-12 in patients with malignant brain tumor, breast cancer, gastric cancer, colorectal carcinoma, ovarian carcinoma, and melanoma [21, 22, 28, 29]. No significant treatment-related toxicities were observed. Clinical responses to the treatment were classified as one of the following four categories: complete response, defined as disappearance of the entire tumor; partial response, defined as a reduction of 50% or more in tumor size; no change (NC), defined as either a decrease of less than 50% or an increase of less than 25% in tumor size; and progressive disease, defined as an increase of 25% or more in tumor size. Three out of 12 patients with malignant brain tumor (25%) achieved a partial response [28], but other patients showed no tumor regression [21]. Thirteen out of 16 patients with brain tumor (81%) showed cutaneous delayed typed hypersensitivity responses [28]. Vaccination of DC/tumor fusion cell and rhIL-12 induced no serious adverse reactions and provided good therapeutic responses in some of the patients with brain tumor. We used extremely small amounts of DC/tumor vaccines and IL-12, but still produced positive results in some patients with malignant brain tumor. Moreover, vaccination with DC/tumor fusion cells and rhIL-12 could potently induce tumor-specific immune responses [21, 28]. Our findings demonstrate that DC/tumor fusion cell vaccination of cancer patients is a feasible, nontoxic approach associated with the induction of immunological and clinical antitumor responses. DC/tumor fusion-based immunotherapy may work more effectively in patients in the early stage of the disease with low tumor burden after surgery, chemotherapy, or irradiation, and patients with a still uncompromised immune system are expected to respond best to this vaccine.

CONCLUSION

DC vaccines represent a promising immunotherapeutic strategy for patients with cancer. DC/tumor fusion can potently induce antitumor immunity in preclinical mice studies and human clinical trials, but has not yet been standardized. Future studies will be needed to examine enhancing vaccine potency.

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REFERENCES

1. Akasaki Y., Kikuchi T., Homma S., Abe T., Kofe D. and Ohno T. (2001): Antitumor effect of immunizations with fusions of dendritic and glioma cells in a mouse brain tumor model. J. Immunother., **24**, 106–113.
2. Avigan D., Vasir B., Gong J., Borges V., Wu Z., Uhl L., Atokins M., Mier J., McDermott D., Smith T., Giallambardo N., Stone C., Schadt K., Dolgoff J., Tetreault J. C., Villarroel M. and Kufe D. (2004): Fusion cell vaccination of patients with metastatic breast and renal cancer induces immunological and clinical responses. Clin. Cancer Res., **10**, 4699–4708.
3. Banchereau J. and Steinman R. M. (1998): Dendritic cells and the control of immunity. Nature, **392**, 245–252.
4. Barry M. and Bleackley R. C. (2002): Cytotoxic T lymphocytes: all roads lead to death. Nat. Rev. Immunol., **2**, 401–409.
5. Cao X., Zhang W., Wang J., Zhang M., Huang X., Hamada H. and Chen W. (1999): Therapy of established tumour with a hybrid cellular vaccine generated by using granulocyte-macrophage colony-stimulating factor genetically modified dendritic cells. Immunology, **97**, 616–625.
6. Cella M., Sallusto F. and Lanzavecchia A. (1997): Origin, maturation and antigen presenting function of dendritic cells. Curr. Opin. Immunol., **9**, 10–16.
7. Chen D., Xia J., Tanaka Y., Chen H., Koido S., Wernet O., Mukherjee P., Gendler S. J., Kufe D. and Gong J. (2003): Immunotherapy of spontaneous mammary carcinoma with fusions of dendritic cells and mucin 1-positive carcinoma cells. Immunology, **109**, 300–307.
8. Du Y. C., Lin P., Zhang J., Lu Y. R., Ning Q. Z. and Wang Q. (2006): Fusion of CpG-ODN-stimulating dendritic cells with Lewis lung cancer cells can enhance anti-tumor immune responses. Tissue Antigens, **67**, 368–376.
9. Fabre J. W. (2001): The allogeneic response and tumor immunity. Nat. Med., **7**, 649–652.
10. Forster R., Schubel A., Breitfeld D., Kremmer E., Renner-Muller I., Wolf E. and Lipp M. (1999): CCR7 coordinates the primary immune responses by establishing functional microenvironments in secondary lymphoid organs. Cell, **99**, 23–33.
11. Gong J., Avigan D., Chen D., Wu Z., Koido S., Kashiwaba M. and Kufe D. (2000): Activation of antitumor cytotoxic T lymphocytes by fusions of human dendritic cells and breast carcinoma cells. Proc. Natl. Acad. Sci. USA, **97**, 2715–2718.
12. Gong J., Chen D., Kashiwaba M. and Kufe D. (1997): Induction of antitumor activity by immunization with fusions of dendritic and carcinoma cells. Nat. Med., **3**, 558–561.
13. Gong J., Chen D., Kashiwaba M., Li Y., Chen L., Takeuchi H., Qu H., Rowse G. J., Gendler S. J. and Kufe D. (1998): Reversal of tolerance to human MUC1 antigen in MUC1

transgenic mice immunized with fusions of dendritic and carcinoma cells. Proc. Natl. Acad. Sci. USA, **95**, 6279–6283.
14. Gong J., Koido S., Chen D., Tanaka Y., Huang L., Avigan D., Anderson K., Ohno T. and Kufe D. (2002): Immunization against murine multiple myeloma with fusions of dendritic and plasmacytoma cells is potentiated by interleukin 12. Blood, **99**, 2512–2517.
15. Gong J., Koido S., Kato Y., Tanaka Y., Chen D., Jonas A., Galinsky I., DeAngelo D., Avigan D., Kufe D. and Stone R. (2004): Induction of anti-leukemic cytotoxic T lymphocytes by fusion of patient-derived dendritic cells with autologous myeloblasts. Leuk. Res., **28**, 1303–1312.
16. Gong J., Nikrui N., Chen D., Koido S., Wu Z., Tanaka Y., Cannistra S., Avigan D. and Kufe D. (2000): Fusions of human ovarian carcinoma cells with autologous or allogeneic dendritic cells induce antitumor immunity. J. Immunol., **165**, 1705–1711.
17. Guo G., Chen S., Zhang J., Luo L., Yu J., Dong H., Xu H., Su Z. and Wu L. (2005): Antitumor activity of a fusion of esophageal carcinoma cells with dendritic cells derived from cord blood. Vaccine, **23**, 5225–5230.
18. Heath W. R. and Carbone F. R. (2001): Cross-presentation in viral immunity and self-tolerance. Nat. Rev. Immunol., **1**, 126–134.
19. Hiraoka K., Yamamoto S., Otsuru S., Nakai S., Tamai K., Morishita R., Ogihara T. and Kaneda Y. (2004): Enhanced tumor-specific long-term immunity of hemagglutinating virus of Japan-mediated dendritic cell-tumor fused cell vaccination by coadministration with CpG oligodeoxynucleotides. J. Immunol., **173**, 4297–4307.
20. Holmes L. M., Li J., Sticca R. P., Wagner T. E. and Wei Y. (2001): A rapid, novel strategy to introduce tumor cell-specific cytotoxic T lymphocyte responses using instant dendritomas. J. Immunother., **24**, 122–129.
21. Homma S., Kikuchi T., Ishiji N., Ochiai K., Takeyama H., Saotome H., Sagawa Y., Hara E., Kufe D., Ryan J. L., Ohno T. and Toda G. (2005): Cancer immunotherapy by fusion of dendritic and tumor cells and rh-IL-12. Eur. J. Clin. Invest., **35**, 279–286.
22. Homma S., Sagawa Y., Ito T., Ohno T. and Toda G. (2006): Cancer immunotherapy using dendritic/tumor-fusion vaccine induces elevation of serum anti-nuclear antibody with better clinical responses. Clin. Exp. Immunol., **144**, 41–47.
23. Homma S., Toda G., Gong J., Kufe D. and Ohno T. (2001): Preventive antitumor activity against hepatocellular carcinoma (HCC) induced by immunization with fusions of dendritic cells and HCC cells in mice. J. Gastroenterol., **36**, 764–771.
24. Hosken N. A. and Bevan M. J. (1992): An endogenous antigenic peptide bypasses the class I antigen presentation defect in RMA-S. J. Exp. Med., **175**, 719–729.
25. Iinuma H., Okinaga K., Fukushima R., Inaba T., Iwasaki K., Okinaga A., Takahashi I. and Kaneko M. (2006): Superior protective and therapeutic effects of IL-12 and IL-18 gene-transduced dendritic neuroblastoma fusion cells on liver metastasis of murine neuroblastoma. J. Immunol., **176**, 3461–3469.
26. Iinuma T., Homma S., Noda T., Kufe D., Ohno T. and Toda G. (2004): Prevention of gastrointestinal tumors based on adenomatous polyposis coli gene mutation by dendritic cell vaccine. J. Clin. Invest., **113**, 1307–1317.
27. Irie M., Homma S., Komita H., Zeniya M., Kufe D., Ohno T. and Toda G. (2004): Inhibition of spontaneous development of liver tumors by inoculation with dendritic cells loaded with hepatocellular carcinoma cells in C3H/HeNCRJ mice. Int. J. Cancer, **111**, 238–245.
28. Kikuchi T., Akasaki Y., Abe T., Fukuda T., Saotome H., Ryan J. L., Kufe D. W. and Ohno T. (2004): Vaccination of glioma patients with fusions of dendritic and glioma cells and recombinant human interleukin 12. J. Immunother., **27**, 452–459.
29. Kikuchi T., Akasaki Y., Irie M., Homma S., Abe T. and Ohno T. (2001): Results of a phase I clinical trial of vaccination of glioma patients with fusions of dendritic and glioma cells. Cancer Immunol. Immunother., **50**, 337–344.
30. Klein J. and Sato A. (2000): The HLA system. First of two parts. N. Engl. J. Med., **343**, 702–709.
31. Koido S., Hara E., Homma S., Torii A., Mitsunaga M., Yanagisawa M., Toyama Y., Kawahara H., Watanabe M., Yoshida S., Kobayashi S., Yanaga K., Fujise K. and Tajiri H. (2007): Streptococcal preparation OK-432 promotes fusion efficiency and enhances induction of antigen-specific CTL by fusions of dendritic cells and colorectal cancer cells. J. Immunol., **178**, 613–622.
32. Koido S., Hara E., Homma S., Torii A., Toyama Y., Kawahara H., Watanabe M., Yanaga K., Fujise K., Tajiri H., Gong J. and Toda G. (2005): Dendritic cells fused with allogeneic colorectal cancer cell line present multiple colorectal cancer-specific antigens and induce antitumor immunity against autologous tumor cells. Clin. Cancer Res., **11**, 7891–7900.
33. Koido S., Hara E., Torii A., Homma S., Toyama Y., Kawahara H., Ogawa M., Watanabe M., Yanaga K., Fujise K., Gong J. and Toda G. (2005): Induction of antigen-specific CD4 and CD8 mediated T cell responses by fusion of autologous dendritic cells and metastatic colorectal cancer cells. Int. J. Cancer, **117**, 587–595.
34. Koido S., Kashiwaba M., Chen D., Gendler S., Kufe D. and Gong J. (2000): Induction of antitumor immunity by vaccination of dendritic cells transfected with MUC1 RNA. J. Immunol., **165**, 5713–5719.
35. Koido S., Nikrui N., Ohana M., Xia J., Tanaka Y., Liu C., Durfee J., Lerner A. and Gong J. (2005): Assessment of fusion cells from patient-derived ovarian carcinoma cells and dendritic cells as a vaccine for clinical use. Gynecol. Oncol., **99**, 462–471.
36. Koido S., Ohana M., Liu C., Nikrui N., Durfee J., Lerner A. and Gong J. (2004): Dendritic cells fused with human cancer cells: morphology, antigen expression and T cell stimulation. Clin. Immunol., **113**, 261–269.
37. Koido S., Tanaka Y., Chen D., Kufe D. and Gong J. (2002): The kinetics of in vivo priming of CD4 and CD8 T cells by dendritic/tumor fusion cells in MUC1-transgenic mice. J. Immunol., **168**, 2111–2117.
38. Koido S., Tanaka Y., Tajiri H. and Gong J. (2007): Generation and functional assessment of antigen-specific T cells stimulated by fusion of dendritic cells and allogeneic breast cancer cells. Vaccine, **25**, 2610–2619.
39. Kufe D. W. (2000): Smallpox, polio and now a cancer vaccine? Nat. Med., **6**, 252–253.
40. Liu Y., Zhang W., Chan T., Saxena A. and Xiang J. (2002): Engineered fusion hybrid vaccine of IL-4 gene-modified myeloma and relative mature dendritic cells enhances antitumor immunity. Leuk. Res., **26**, 757–763.
41. Mackensen A., Herbst B., Chen J. L., Kohler G., Noppen C., Herr W., Spagnoli G., Cerundolo V. and Lindemann A. (2000): Phase I study in melanoma patients of a vaccine with peptide-pulsed dendritic cells generated *in vitro* from

CD34(+) hematopoietic progenitor cells. Int. J. Cancer, **86**, 385–392.
42. Marzo A. L., Kinnear B. F., Lake R. A., Frelinger J. J., Collins E. J., Robinson B. W. and Scott B. (2000): Tumor-specific CD4+ T cells have a major "post-licensing" role in CTL mediated anti-tumor immunity. J. Immunol., **165**, 6047–6055.
43. Nestle F. O., Alijagic S., Gilliet M., Sun Y., Grabbe S., Dummer R., Burg G. and Schadendorf D. (1998): Vaccination of melanoma patients with peptide- or tumor lysate--pulsed dendritic cells. Nat. Med., **4**, 328–332.
44. Paglia P., Chiodoni C., Rodolfo M. and Colombo M. P. (1996): Murine dendritic cells loaded *in vitro* with soluble protein prime cytotoxic T lymphocytes against tumor antigen *in vivo*. J. Exp. Med., **183**, 317–322.
45. Patton S., Gendler S. J. and Spicer A. P. (1995): The epithelial mucin, MUC1, of milk, mammary gland and other tissues. Biochim. Biophys. Acta, **1241**, 407–423.
46. Rowse G. J., Tempero R. M., VanLith M. L., Hollingsworth M. A. and Gendler S. J. (1998): Tolerance and immunity to MUC1 in a human MUC1 transgenic murine model. Cancer Res., **58**, 315–321.
47. Russo V., Tanzarella S., Dalerba P., Rigatti D., Rovere P., Villa A., Bordignon C. and Traversari C. (2000): Dendritic cells acquire the MAGE-3 human tumor antigen from apoptotic cells and induce a class I-restricted T cell response. Proc. Natl. Acad. Sci. USA, **97**, 2185–2190.
48. Savai R., Schermuly R. T., Schneider M., Pullamsetti S. S., Grimminger F., Seeger W. and Banat G. A. (2006): Hybrid-primed lymphocytes and hybrid vaccination prevent tumor growth of Lewis lung carcinoma in mice. J. Immunother., **29**, 175–187.
49. Sigal L. J., Crotty S., Andino R. and Rock K. L. (1999): Cytotoxic T-cell immunity to virus-infected non--haematopoietic cells requires presentation of exogenous antigen. Nature, **398**, 77–80.
50. Steinman R. M. (1991): The dendritic cell system and its role in immunogenicity. Annu. Rev. Immunol., **9**, 271–296.
51. Steinman R. M. and Cohn Z. A. (1973): Identification of a novel cell type in tissue distribution. J. Exp. Med., **137**, 1142–1162.
52. Steinman R. M., Hawiger D. and Nussenzweig M. C. (2003): Tolerogenic dendritic cells. Annu. Rev. Immunol., **21**, 685–711.
53. Tanaka Y., Koido S., Chen D., Gendler S. J., Kufe D. and Gong J. (2001): Vaccination with allogeneic dendritic cells fused to carcinoma cells induces antitumor immunity in MUC1 transgenic mice. Clin. Immunol., **101**, 192–200.
54. Tanaka Y., Koido S., Ohana M., Liu C. and Gong J. (2005): Induction of impaired antitumor immunity by fusion of MHC class II-deficient dendritic cells with tumor cells. J. Immunol., **174**, 1274–1280.
55. Tanaka Y., Koido S., Xia J., Ohana M., Liu C., Cote G. M., Sawyer D. B., Calderwood S. and Gong J. (2004): Development of antigen-specific CD8+ CTL in MHC class I-deficient mice through CD4 to CD8 conversion. J. Immunol., **172**, 7848–7858.
56. Thurner B., Haendle I., Roder C., Dieckmann D., Keikavoussi P., Jonuleit H., Bender A., Maczek C., Schreiner D., von den Driesch P., Brocker E. B., Steinman R. M., Enk A., Kampgen E. and Schuler G. (1999): Vaccination with Mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. J. Exp. Med., **190**, 1669–1678.
57. Wang J., Saffold S., Cao X. T., Krauss J. and Chen W. (1998): Eliciting T cell immunity against poorly immunogenic tumors by immunization with dendritic cell-tumor fusion vaccines. J. Immunol., **161**, 5516–5524.
58. Xia J., Tanaka Y., Koido S., Liu C., Mukherjee P., Gendler S. J. and Gong J. (2003): Prevention of spontaneous breast carcinoma by prophylactic vaccination with dendritic/ /tumor fusion cells. J. Immunol., **170**, 1980–1986.