Introduction

Dendritic cells (DCs) operate as professional antigen-presenting cells (APCs) and play a critical role in the induction of immune responses against pathogens and malignant cells. Thus, DC-based anticancer vaccines are being intensively investigated, in both preclinical and clinical settings. To obtain tumor-specific vaccines, DCs have been loaded with tumor-associated antigens (TAAs) in the form of purified peptides, malignant cell lysates or apoptotic cancer cells, as well as upon transfection with TAA-encoding mRNAs. An alternative strategy for inducing DC-dependent antitumor immune responses relies on the fusion of DCs and whole cancer cells (Fig. 1). This approach allows DCs to express (and hence present to T cells in the context of MHC molecules) the entire repertoire of TAAs contained in fused cancer cells. In preclinical models, DC/malignant cell fusions have been shown to possess all the elements that are required for the processing of TAAs and their presentation to immune cells, resulting in the elicitation of effective antitumor immune responses that were able to break peripheral T-cell tolerance to TAAs.8,9

Stimulation of T Cells by DC/Cancer Cell Fusion-Based Anticancer Vaccines

Upon fusion, the cytoplasmic compartments of DCs and cancer cells mix, while their nuclei remain separate. Such a peculiar configuration allow DC/cancer cell fusion to maintain the functions of both parental cell types (Fig. 1). Therefore, within DC/malignant cell fusions, TAAs (be they known or unidentified) efficiently feed into the antigen-processing pathway and antigenic TAA-derived peptides are presented on cell surface in complex with MHC class I or II molecules and in the presence of co-stimulatory factors (Fig. 1). Moreover, DC/cancer cell fusions are able to migrate to tumor-draining lymph nodes, where they can directly interact with CD4+ and CD8+ T cells, thus inducing robust antitumor immune responses. This is important as the direct presentation of TAAs by DC/malignant cell fusions can bypass the defects in the APC compartment often manifested by cancer patients. Host DCs can also take up TAAs released from dying DC/cancer cell fusions and represent them on MHC class I and II molecules to simultaneously activate CD4+ and CD8+ T cells. Thus, TAA-specific T cells can be induced by DC/neoplastic cells fusions either directly or indirectly.

Immunogenicity of DC/Malignant Cell Fusions as Anticancer Vaccines

Despite the unique features of DC/cancer cell fusions described above and their ability to promote tumor eradication in animal models, limited, yet encouraging, success has been obtained with this immunotherapeutic approach in clinical trials. In murine tumor models, many adjuvants, including interleukin (IL)-2, IL-12 and IL-18, as well as synthetic oligodeoxynucleotides (ODNs) containing unmethylated CpG motifs, have been shown to improve the immunogenicity of DC/malignant cell fusions as anticancer vaccines.

The rationale for fusing dendritic cells (DCs) with whole tumor cells to generate anticancer vaccines resides in the fact that the former operate as potent antigen-presenting cells, whereas the latter express a constellation of tumor-associated antigens (TAAs). Although the administration of DC/malignant cell fusions to cancer patients is safe and this immunotherapeutic intervention triggers efficient tumorspecific T-cell responses in vitro, a limited number of objective clinical responses to DC/cancer cell fusions has been reported thus far. This review discusses novel approaches to improve the immunogenicity of DC/malignant cell fusions as anticancer vaccines.

Keywords: dendritic cell, whole tumor cell, immunogenicity, cytotoxic T lymphocyte, fusion

Strategies to improve the immunogenicity of anticancer vaccines based on dendritic cell/malignant cell fusions

Shigeo Koido1,2,3,*, Sadamu Homma1, Masato Okamoto4, Yoshihisa Namiki2, Kazuki Takakura1, Kan Uchiyama1, Mikio Kajihara1, Seiji Arihiro1, Hiroo Imazu5, Hiroshi Arakawa1,2, Shin Kan1, Hideo Komita3, Yuko Kamata4, Masaki Ito1, Toshifumi Ohkusa1, Jianlin Gong6, and Hisao Tajiri1

1Division of Gastroenterology and Hepatology; Department of Internal Medicine; The Jikei University School of Medicine; Tokyo, Japan; 2Institute of Clinical Medicine and Research; The Jikei University School of Medicine; Tokyo, Japan; 3Department of Oncology; Institute of DNA Medicine; The Jikei University School of Medicine; Tokyo, Japan; 4Division of Cellular Signaling; Institute for Advanced Medical Research; Keio University School of Medicine; Tokyo, Japan; 5Department of Endoscopy; The Jikei University School of Medicine; Tokyo, Japan; 6Department of Medicine; Boston University School of Medicine; Boston, MA USA

1Correspondence to: Shigeo Koido; Email: shigeo_koido@jikei.ac.jp
Submitted: 07/21/2013; Accepted: 07/31/2013
Citation: Homma S, Okamoto M, Namiki Y, Takakura K, Uchiyama K, Kajihara M, Arihiro S, Imazu H, Arakawa H, Kan S, et al. How to improve the immunogenicity of dendritic/tumor fusion cells as cancer vaccines. OncolImmunology 2013; 2:e25994; http://dx.doi.org/10.4161/onci.25994

www.landesbioscience.com OncolImmunology e25994-1
motifs and polyinosinic-polycytidylic acid (polyI:C), functioning as agonists for Toll-like receptor 9 (TLR) and TRL3, respectively, have been combined with DC/malignant cell fusions to evoke antitumor immune responses. Therefore, adjuvants may be essential to boost antitumor immunity when DC/neoplastic cell fusion-based vaccines are used to treat patients with advanced cancer. Indeed, the administration of these vaccines to cancer patients has been associated with immunological responses. Early clinical trials have shown limited rates of success for this immunotherapeutic approach. Optimal responses to DC/malignant cell fusion-based anticancer vaccines may therefore require maneuvers that exacerbate the immunogenicity of both DCs and neoplastic cells.

**Improving the Immunogenicity of DCs**

The clinical outcome of tumor-specific immune responses developing in cancer patients receiving DC/malignant cell fusions is significantly influenced by the characteristics of the DCs employed for the creation of the vaccine. TLRs have recently emerged as key components of the innate immune system, triggering DC activation and the secretion of pro-inflammatory cytokines in response to a wide panel of conserved microbial or endogenous components. In line with notion, the co-administration of TLR agonists with anticancer vaccines modulates the activity of regulatory T cells (Tregs) and DCs through numerous mechanisms: (1) the activation of DCs by TLR agonists favors the exposure on the cell surface of antigenic peptides complexed with MHC molecules, the expression of co-stimulatory molecules (e.g., CD80 and CD86) as well as the secretion of IL-12; (2) DCs activated by TLR agonists render naïve T cells refractory to Treg-dependent immunosuppression; (3) TLR agonists activate DCs at the tumor site, thus enhancing antigen cross-presentation as well as DC migration to regional lymph nodes, and hence promoting antigen-specific cytotoxic T lymphocyte (CTL) responses; and (4) TLR agonists such as CpG ODNs can...
preventing the activation-induced death of CTLs by increasing the expression of anti-apoptotic molecules such as the BCL-2 family members BCL-XL, and CASP8 and FADD-like apoptosis regulator (CFLAR, best known as cFLIP), thereby allowing CTLs to survive and reach neoplastic lesions. However, the administration of a single TLR agonist has been reported to increase the expression of only approximately 1% of gene transcripts, a phenomenon that was exacerbated by more than 5-fold upon synergistic TLR stimulation. Thus, the optimal activation of DCs may require the engagement of multiple TLR-dependent signaling pathways. We and others have previously reported that the co-administration of various TLR agonists promotes the immunogenicity of DC/malignant cell fusions through the upregulation of IL-12. In this setting, we used a protein-bound polysaccharide isolated from Coriolus versicolor (PSK, which operates as a TLR2 agonist) and lyophilized preparations of a low-virulence strain (Su) of Streptococcus pyogenes (OK-432, which acts as a TLR4 agonist), both of which can be produced as good manufacturing practice (GMP)-grade agents and have been previously used in the clinic as biological response modifiers. Of note, DC/cancer cell fusions activated in the presence of both TLR2 and TLR4 agonists, but not DC/malignant cell fusions that were left unstimulated or were exposed to either TLR agonist alone, overcame the immunosuppressive activity of tumor-derived molecules such as transforming growth factor β1 (TGFβ1). In particular, TLR2/4-activated DCs (or the corresponding fusions): (1) exhibit increased expression levels of MHC class II molecules and CD86 on the cell surface; (2) manifest an improved fusion efficacy; (3) produce elevated levels of IL-12; (4) simultaneously activate CD4+ and CD8+ T cells, which secrete high levels of interferon γ (IFNγ); (5) potently induce antigen-specific CTL activity; and (6) manifest a superior efficacy in inhibiting the generation of CD4+CD25+FoxP3+ Tregs. Nonetheless, when DC/cancer cell fusions are generated with neoplastic cells producing extremely high levels of TGFβ1, they inhibit the activity of CTLs in vitro. Therefore, incorporating the simultaneous activation of multiple TLRs and the blockade of immunosuppressive that are intrinsically produced by DC/neoplastic cell fusions may significantly enhance the therapeutic potential of this approach.

Improving the Immunogenicity of Malignant Cells

Most, if not all, malignant cells secrete multiple immunosuppressive mediators such as TGFβ1, IL-10 and vascular endothelial growth factor (VEGF). As these immunosuppressive molecules normally inhibit the initiation of efficient CTL responses, the microenvironment of malignant cells used for the generation of DC/cancer cell fusions has to be rendered immunostimulatory. Several strategies to inhibit the production of immunosuppressive factors by cancer cells have been developed, including the administration of neutralizing antibodies and small chemical inhibitors, as well as the transfection of specific small-interfering RNAs (siRNAs) or constructs coding for a soluble variant of the TGFβ receptor. Also heat-shock proteins (HSPs), which have recently been implicated in the immunogenicity of apoptotic and necrotic cells, might constitute effective adjuvant for boosting the efficacy of DC/neoplastic cell fusions. HSPs generally operate as chaperons for a wide panel of peptides, including antigenic peptides, and HSP/peptide complexes not only can be efficiently taken up by DCs through specific receptors, but also can be presented in complex with MHC class I and II molecules the DC surface. We have previously reported that TLR2-stimulated DCs fused with heat-treated cancer cells are immunogenic, as demonstrated by: (1) the upregulation of multiple HSPs, MHC class I and II molecules, TAAs, CD80, CD86, CD83, and IL-12; (2) their ability to activate CD4+ and CD8+ T cells producing high levels of IFNγ; and (3) the capacity to efficiently elicit antigen-specific CTL activity. More recently, we have demonstrated that the secretion of TGFβ1, IL-10 and VEGF from whole cancer cells is significantly limited upon exposure to pharmaceutical grade ethanol, a maneuver that does not reduce the levels of MHC class I molecules and TAAs on the cell surface. Moreover, ethanol, employed at concentrations that affect tumor growth, promoted the upregulation of HSPs. HSPs exposed by cancer cells can be recognized by DCs via TLR4, facilitating their activation and promoting antigen processing and presentation.

Of note, malignant cells that undergo immunogenic apoptosis ectopically expose the Ca2+-binding chaperone calreticulin (CRT) on the cell surface, allowing TAAs to efficiently traffic to the DC antigen-presenting compartment. Moreover, high-mobility group box 1 (HMGB1) passively released from dying neoplastic cells can stimulate antigen processing and presentation in DCs via a TLR4-dependent signaling pathway. Therefore, the exposure of CRT and the release of HMGB1 by ethanol-treated malignant cells enhance the immunogenicity of DC/cancer cell fusions. Importantly, fusions involving DCs and ethanol-treated cancer cells activate T cells to produce high levels of IFNγ, boosting the elicitation of antigen-specific CTL response in vitro. In addition, HSP70-peptide complexes derived from DC/cancer cell fusions appear to possess superior immunogenic properties as compared with similar complexes obtain from neoplastic cells.

Synergistic Effects of Fusions Generated with Immunogenic DCs and Cancer Cells

One of the biggest advantages of DC/malignant cell fusion-based anticancer vaccines is that DCs and neoplastic cells can be modified independently from each other (before fusion), but these alterations persist for long period (after fusion). This is an important difference between this approach and the use of DCs loaded with cancer cell lysates. Thus, fusing TLR-activated DCs with cancer cells that express abundant danger signals, including HSPs, may result in efficient antigen processing and presentation in the context of high levels of MHC molecules and co-stimulatory factors. We have recently reported that improved CTL responses are induced by DC/cancer cell fusions generated with neoplastic cells expressing danger/alarm signals and DCs stimulated with TLR4 agonists in vitro. However, it is still unclear which among multiple treatments that induce the immunogenic demise of neoplastic cells, including cytotoxic chemotherapy, targeted anticancer drugs and ionizing irradiation, and which (combination of) TLR agonists must be
harnessed to obtain optimal DC/malignant cell fusion-based anticancer vaccines.

**Conclusions**

Even upon a significant improvement of their immunogenicity, DC/malignant cell fusion-based anticancer vaccines alone may still be insufficient to generate therapeutically relevant immune responses in patients affected by advanced neoplasms. To circumvent this issue, DC/cancer cell fusions might have to be combined with other forms of immunotherapy or conventional chemotherapy. In murine models, the combination of DC/neoplastic cell fusions with adoptive immunotherapy was very effective against poorly immunogenic tumors.35 As an alternative, the therapeutic profile of DC/cancer cell fusions might be improved upon combination with various strategies that inhibit the immunosuppressive activity of Tregs while promoting CTL responses, including specific types of chemotherapy, radiotherapy, hormonal therapy, photodynamic therapy and immunostimulatory monoclonal antibodies. Importantly, the blockade of immunological checkpoints with monoclonal antibodies specific for programmed cell death 1 (PDCD1, best known as PD-1), its ligands, namely CD274 (also known as PD-L1) and CD273 (also known as PD-L2), or cytotoxic T lymphocyte-associated protein 4 (CTLA4) is emerging as a promising immunotherapeutic strategy against cancer.36–39 All these interventions may be combined with DC/cancer cell fusions to treat cancer patients.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**References**

1. Nestle FO, Alijagic S, Gilliet M, Sun Y, Grabbe S, Dummer R, Burg G, Schadendorf D. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. Nat Med 1998; 4:328-32; PMID:9500607; http://dx.doi.org/10.1038/nm0398-328

2. Mackensen A, Herbst B, Chen JL, Kohler G, Noppen C, Herr W, Spagnoli GC, Cerundolo V, Lindemann A. 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 2000; 86:385-92; PMID:10768827; http://dx.doi.org/10.1002/(SICI)1097-0215(20000501)86:3<385::AID-IJC13>3.0.CO;2-T

3. Palucka AK, Ueno H, Connolly J, Kerneis-Norvell F, Blank JF, Johnston DA, Fay J, Banchereau J. Dendritic cells loaded with killed allogeneic melanoma cells can induce objective clinical responses and MART-1 specific CD8+ T-cell immunity. J Immunother 2006; 29:385-92; PMID:16971810; http://dx.doi.org/10.1097/01.cji.0000211309.90621.8b

4. Nair SK, Boczkowski D, Morse M, Cumming RI, Lyerly HK, Gilboa E. Induction of primary carcinoembryonic antigen (CEA)-specific cytotoxic T lymphocytes in vitro using human dendritic cells transfected with RNA. Nat Biotechnol 1998; 16:364-9; PMID:9555728; http://dx.doi.org/10.1038/nbt0498-364

5. Koido S, Kashihara M, Chen D, Gendler S, Kufe D, Gong J. Induction of antitumor immunity by vaccination of dendritic cells transfected with MUC1 RNA. J Immunol 2000; 165:5713-9; PMID:11067929

6. Gong J, Chen D, Kashihara M, Kufe D. Induction of antitumor activity by immunization with fusions of dendritic and carcinoma cells. Nat Med 1997; 3:558-61; PMID:9342127; http://dx.doi.org/10.1038/nm0597-558
