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The combination of TLR2 and TLR4 agonists promotes the immunogenicity of dendritic cell/cancer cell fusions

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The induction of antitumor immune responses by dendritic cell (DC)/tumor cell fusions can be modulated by their activation status. Our recent work reveals that the combination of Toll-like receptor 2 (TLR2) and TLR4 agonists promotes the immunogenicity of DC/tumor cell fusions, allowing them to overcome the immunosuppressive effects of transforming growth factor β1.

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that can initiate robust $CD8^+$ cytotoxic T-lymphocyte (CTL) immune responses and hence have been extensively used for the development of anticancer vaccines.[1] One of the strategies that have been investigated for the induction of antitumor immunity is administration of DCs fused with whole tumor cells.[2] DC/tumor cell fusions are able to present a broad spectrum of tumor-associated antigens (TAAs), including known as well as unidentified molecules, on MHC class I and class II molecules and in the context of co-stimulatory molecules. Therefore, DC/tumor cell fusion-based vaccines target multiple TAAs at once, generating broad antitumor immune responses that—at least potentially—bypass issues related to tumor antigen loss. Moreover, the use of whole tumor cells theoretically eliminates the need to define, test and select for immunodominant antigenic epitopes.

DC/tumor cell fusions have been shown to possess all the elements required for processing and presenting multiple TAAs to host immune cells, hence inducing effective antitumor immune responses and breaking T-cell tolerance to TAAs, at least in animal models.[3] Although the immunization of advanced cancer patients with DC/tumor cell fusions has been associated with immunological responses in Phase I clinical trials, limited therapeutic benefits have been observed in this setting.[3] As DC/tumor cell fusion-based vaccines have originally been developed in animal models, many adjuvants, including interleukin (IL)-2, IL-12, IL-18, as well as synthetic oligodeoxynucleotides (ODNs) containing specific bacterial unmethylated CpG motifs, have been used to enhance their ability to promote antitumor immune responses. Therefore, adjuvants may be needed to increase the therapeutic potential of DC/tumor cell fusions in cancer patients. Recently, the signals required for the activation of DCs have been intensively investigated, revealing a predominant role for Toll-like receptors (TLRs).[4] The most prominent advantage of DC/tumor cell fusion-based strategies is that DCs and whole tumor cells can be modified independently and these alterations persist after fusion. Therefore, the therapeutic efficacy of DC/tumor cell fusion might benefit from the stimulation of both DCs and whole tumor cells with TLR agonists, de facto improving their immunogenicity.

TLR ligand robustly activate DCs and tumor cells.[4] The ligation of TLR2 and TLR4 by pathogen-associated molecular patterns potently induces DC maturation, leading to the secretion of several cytokines as well as to the upregulation of co-stimulatory molecules. Moreover, TLR2/4 agonists induce the production of tumor necrosis factor α (TNFα) and the expression of inducible nitric oxide (NO) synthase (iNOS) by tumor cells. At least under selected circumstances, TNFα and NO are able to induce the apoptotic demise of chemotherapy-resistant tumor cells.[5] Thus, the use of TLR2/4 agonists may have a dual advantage for the development of DC/tumor cell fusion-based anticancer vaccines. Of note, some TLRs activate a signaling a cascade that promote to the evasion of malignant cells from immunosurveillance.[5] This possibility poses safety issues that must be taken under attentive consideration.

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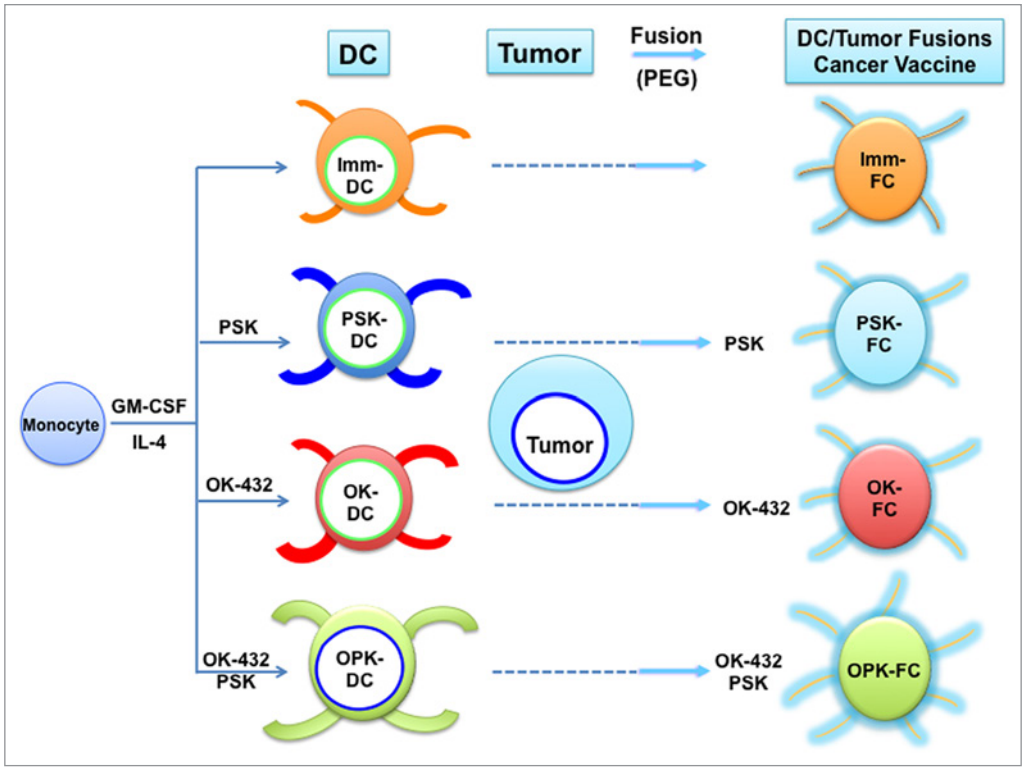

Figure 1. Effects of Toll-like receptor agonists on the immunogenicity of dendritic cell/tumor cell fusions. Dendritic cells (DCs) stimulated with a Toll-like receptor 2 (TLR2) agonist (PSK), a TLR4 agonist (OK-432), or both were fused with whole tumor cells by means of polyethylene glycol (PEG). DC/tumor cell fusions (FCs) were then maintained in the presence of PSK (PSK-FC), OK-432 (OK-FC) or both (OPK-FC). OPK-FCs elicit more robust cytotoxic T-lymphocyte (CTL) responses than PSK-FCs, OK-FCs, and unstimulated FCs.

We have previously reported that fusing TLR4-stimulated DCs with heat-treated tumor cells improves the immunogenicity of DC/tumor cell fusions.[6] However, the administration of a single TLR agonist appears to have limited effects on DCs, as it only induces the expression of approximately 1% of all gene transcripts. Conversely, gene expression is increased by more than 5-fold upon the administration of multiple TLR ligands.[7] Thus, the full-blown activation of DC/tumor cell fusions may require the assembly of receptor signaling complexes by the combination of several TLR agonists. To confirm the hypothesis, we used a protein-bound polysaccharide isolated from *Coriolus versicolor* (PSK, which is a TLR2 agonist) and penicillin-killed *Streptococcus pyogenes* (OK-432, which activates TLR4).[8,9] Indeed, DC/tumor cell fusions stimulated by PSK and OK-432 induced more robust antigen-specific CTL responses in vitro than DC/tumor cell fusions received either TLR agonist alone (**Fig. 1**).[9]

Although the combination of TLR2 and TLR4 agonists promoted the immunogenicity of DC/tumor cell fusions, transforming growth factor β1 (TGFβ1) antagonized this effect. Several types of malignant cells secrete immunosuppressive factors such as TGFβ1, vascular endothelial growth factor (VEGF) and IL-10. Thus, the microenvironment of tumor cells must also be modified to allow DC/tumor cell fusion-based vaccine to elicit antitumor immunity. One of the most effective adjuvants for whole tumor cell-based anticancer vaccines are stress-inducible molecules that have been shown to underlie the ability of apoptotic and necrotic cancer cells to activate tumor-specific immune responses.[6] TGFβ1-blocking strategies may also enhance the efficacy of DC/tumor cell fusion-based anticancer vaccines. As it stands, it is unclear which specific anticancer therapies lead to immunogenic tumor cell death. We are currently searching for optimal ways to maximize the clinical benefits of DC/tumor cell fusion-based anticancer vaccines. The fusion DCs with whole tumor cells genetically modified to express cytokines, chemokines or co-stimulatory molecules may ameliorate the CTL responses triggered by DC/tumor cell fusions. Finally, combinatorial approaches involving conventional anticancer therapies appear as mandatory to successfully prolong avoid disease relapse and prolong patient survival. In particular, the combination of DC/tumor cell fusions with specific chemo-, radio-, or immunotherapeutic regimens may stimulate protective anticancer immune responses that overcome local immunosuppression and hence provide robust clinical benefits to cancer patients.[10]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Steinman RM, Banchereau J. Taking dendritic cells into medicine. Nature 2007; 449:419-26; PMID:17898760; http://dx.doi.org/10.1038/nature06175
2. Gong J, Chen D, Kashiwaba M, Kufe D. Induction of antitumor activity by immunization with fusions of dendritic and carcinoma cells. Nat Med 1997; 3:558-61; PMID:9142127; http://dx.doi.org/10.1038/nm0597-558
3. Koido S, Hara E, Homma S, Namiki Y, Ohkusa T, Gong J, et al. Cancer vaccine by fusions of dendritic and cancer cells. Clin Dev Immunol 2009; 2009:657369; PMID:20182533; http://dx.doi.org/10.1155/2009/657369
4. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol 2001; 2:675-80; PMID:11477402; http://dx.doi.org/10.1038/90609
5. Yu L, Chen S. Toll-like receptors expressed in tumor cells: targets for therapy. Cancer Immunol Immunother 2008; 57:1271-8; PMID:18256828; http://dx.doi.org/10.1007/s00262-008-0459-8
6. Koido S, Hara E, Homma S, Mitsunaga M, Takahara A, Nagasaki E, et al. Synergistic induction of antigen-specific CTL by fusions of TLR-stimulated dendritic cells and heat-stressed tumor cells. J Immunol 2007; 179:4874-83; PMID:17878387
7. Napolitani G, Rinaldi A, Bertoni F, Sallusto F, Lanzavecchia A. Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. Nat Immunol 2005; 6:769-76; PMID:15995707; http://dx.doi.org/10.1038/ni1223
8. Koido S, Hara E, Homma S, Torii A, Mitsunaga M, Yanagisawa S, et al. 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 2007; 178:613-22; PMID:17182602
9. Koido S, Homma S, Okamoto M, Namiki Y, Takakura K, Takahara A, et al. Combined TLR2/4-Activated Dendritic/Tumor Cell Fusions Induce Augmented Cytotoxic T Lymphocytes. PLoS One 2013; 8:e59280; PMID:23555011; http://dx.doi.org/10.1371/journal.pone.0059280
10. Koido S, Homma S, Takahara A, Namiki Y, Komita H, Uchiyama K, et al. Immunotherapy synergizes with chemotherapy targeting pancreatic cancer. Immunotherapy 2012; 4:5-7; PMID:22149993; http://dx.doi.org/10.2217/imt.11.150