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Universal tumor-reactive helper peptides from telomerase as new tools for anticancer vaccination

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Accumulating evidence demonstrates the importance of CD4$^+$ T cells in antitumor immune responses. Identifying promiscuous MHC Class II-binding peptides derived from relevant tumor-associated antigens that specifically target CD4$^+$ helper T cells in vivo represent a powerful approach to fully exploit these cells for anticancer immunotherapy.

Recent advances indicate that adaptive immune responses play a critical role in cancer immunosurveillance. Among various cell types involved in adaptive immunity, CD4$^+$ helper T-cell subpopulations are critical for antitumor immune response.[1] In particular, tumor-reactive CD4$^+$ T helper 1 cells (T_H1) produce cytokines such as interferon γ (IFNγ), tumor necrosis factor α (TNFα) and interleukin (IL-)2, which are essential for the induction of cell-mediated immunity against tumors. Thus, CD4$^+$ T_H1 cells play a key role in "helping" antigen-specific CD8$^+$ T cells to efficiently undergo activation and proliferation. According to a commonly accepted model, CD4$^+$ T cells license dendritic cells (DCs) for efficient CD8$^+$ T-cell priming through the interaction of co-stimulatory receptors such as CD40 with their ligands (e.g., CD40L).[2] Supporting the critical role of CD4$^+$ T cells in antitumor immunity, a high density of tumor-infiltrating T_H1 cells has been shown to constitute a good prognostic marker in several cancers.[3] Hence, there is a growing interest to specifically stimulate CD4$^+$ T_H1 cells for cancer immunotherapy.

We have recently described four MHC Class II-restricted peptides derived from human telomerase reverse transcriptase (TERT). TERT is a prototype of universal tumor-associated antigen, as it is overexpressed by the vast majority of human cancers and appears as an attractive target for anticancer immunotherapy.[4] These novel peptides, which we referred to as "Universal Cancer Peptides" (UCPs), efficiently bind various HLA-DR molecules, increasing their likelihood to be immunogenic in a large number of patients.[5] Such a promiscuous binding capacity of UCPs circumvents one major limitation of the clinical use of tumor-derived peptides, which only bind a few MHC Class II molecules.

In a recent report, UCPs were used to actively target CD4$^+$ T_H1 cells in vivo and the helper properties of UCP-specific CD4$^+$ T cells were systematically analyzed in a preclinical tumor model.[6] Using the HLA-A2/HLA-DR1 transgenic mouse model, we showed that vaccinations with UCPs induce high-avidity specific CD4$^+$ T_H1 responses. These UCP-specific CD4$^+$ T cells produce high amount of IFNγ and IL-2 and but not IL-4, IL-5, IL-10 and IL-17. The immunization of mice with MHC Class I-restricted tumor peptide alone or combined with UCP showed that UCP-specific CD4$^+$ T cells fulfill the helper features that are necessary to generate potent cellular antitumor responses. Indeed, the addition of UCPs as helper peptides drastically increased tumor-specific cytotoxic T lymphocyte (CTL) responses and mice survival. The magnitude and quality of CTL responses were closely correlated with the strength of UCP-specific CD4$^+$ T_H1 responses. The activation of DCs was also found to be increased in vivo following vaccination with UCPs and in vitro after the co-culture of immature DCs with UCP-specific CD4$^+$ T cells.

We demonstrated that the mechanism underpinning the upregulation of activation markers such as CD86 and MHC Class II on DCs involves both the secretion of IFNγ and granulocyte-macrophage colony-stimulating factor (GM-CSF) and the expression of CD40L by UCP-specific CD4$^+$ T cells. Finally, by using a model of transplantable mouse melanoma (B16-HLA-A2 cells), we showed that the addition of UCPs to a MHC Class I peptide-based therapeutic vaccination is necessary to promote tumor regression by fostering the recruitment of tumor-specific CD8$^+$ effector T cells at the tumor site. Altogether, these results indicate that the stimulation of UCP-specific CD4$^+$ T_H1 cells may constitute a powerful

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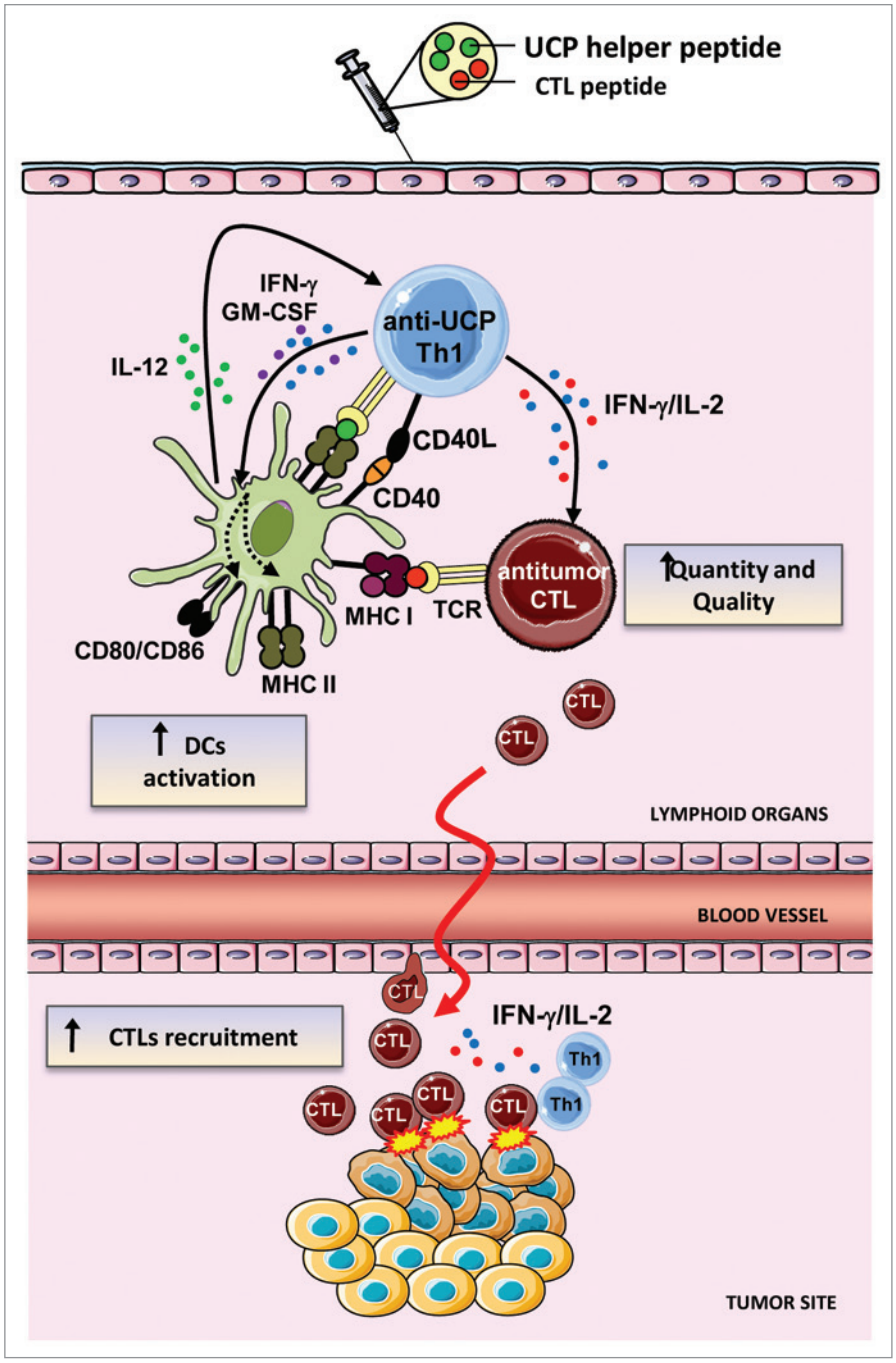

Figure 1. Cellular effects of universal cancer peptides-based antitumor vaccination. CTL, cytotoxic T lymphocyte; DC, dendritic cell; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFNγ, interferon γ; IL, interleukin; TCR, T-cell receptor; T_H1; T helper 1; UCP, universal cancer peptides.

method to improve the efficiency of anticancer vaccines (**Fig. 1**).

During the past ten years, great interest has been attracted by the characterization of immunogenic helper epitopes derived from tumor-associated antigens to actively target $CD4^+$ T cells.[7] However, the use of tumor-reactive $CD4^+$ T cell-targeting peptides requires particular caution to prevent the induction of detrimental immune responses, as different subpopulations of $CD4^+$ T_H cells are known to regulate host antitumor immune responses. For instance, T_H2 and regulatory T cells are frequently associated with the establishment of an immunosuppressive environment within the tumor, whereas the role of T_H17 cells is still controversial and seems to vary, at least in part, with cancer type.[1,3] Thus, only T_H1 immune response have been shown to mediate bona fide anticancer effects, providing a strong rationale to develop anticancer vaccines that stimulate antitumor T_H1 immunity. Nevertheless, only few tumor-reactive $CD4^+$ T cell-targeting peptides are currently used in clinical settings, and in most cases the helper features of these peptides had not been evaluated before their clinical deployment. Another critical consideration is the nature of helper peptides used for anticancer vaccination. The synthetic helper peptide PADRE, which is derived from keyhole limpet hemocyanin (KLH), and the tetanus toxoid-derived helper peptide are commonly used in anticancer vaccines although they have never provided a real impact on disease outcome, as exemplified in a recent study involving melanoma patients.[8] In this clinical trial, patients were vaccinated with MHC Class I-restricted tumor-derived peptides in conjunction with either helper peptides derived from melanoma antigens or the tetanus toxoid-derived helper peptide. Although more robust $CD8^+$ T-cell responses were induced in patients receiving the tetanus toxoid-derived helper peptide than in individuals getting the melanoma-derived helper peptide, the clinical outcome was relatively similar in the two groups. One possible explanation for these observations is that helper peptides unrelated to tumor antigens may be ineffective in guiding effector $CD8^+$ T cells within the tumor, as recently demonstrated by Sherman and colleagues.[9,10] Indeed, only $CD4^+$ T cells specific for tumor-associated antigens are effectively able to pave the way for the entry of CTLs within the tumor. In view of these findings, our results provide a robust method to comprehensively analyze tumor-derived helper peptides for anticancer vaccines.

In conclusion, there is great interest to stimulate antitumor T_H1 responses by using universal tumor-reactive helper peptides such as TERT-derived UCPs, as these may potentially be applied to several distinct types of cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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