OncoImmunology 2:9, e25801; September 2013; © 2013 Landes Bioscience

Long peptide-based cancer immunotherapy targeting tumor antigen-specific CD4+ and CD8+ T cells

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Keywords: cDNA microarray analysis, cross-presentation, helper T-cell epitope, peptide vaccine, Type 1 helper T cell, tumor-associated antigen

CD4+ T cells promote cytotoxic T lymphocyte (CTL)-mediated anticancer immune responses. We have recently identified ideal tumor-associated antigen (TAA)-derived long peptides (LPs) that elicit not only TAA-specific T_H1 response, but also CTLs, through cross-presentation. The LP-specific T_H1 cell responses were augmented in cancer patients vaccinated with CTL epitopes. Our findings support the clinical application of LP-based immunotherapy.

The importance of CD4+ T cells in orchestrating the activity of the immune system and inducing effective T cell-mediated anticancer immune responses is indisputable. CD4+ helper T cells are necessary for the priming of tumor-specific CD8+ T cells, influence the differentiation and expansion of tumor-associated antigen (TAA)-specific cytotoxic T lymphocytes (CTLs), and are essential for the generation and maintenance of long-lasting CD8+ T-cell responses. Moreover helper T cells pave the way for the infiltration of neoplastic lesions by CTLs and T_H1 cells not only play a direct role in the elimination of malignant cells but also mediate anti-angiogenic effects, mostly via their ability to secrete interferon γ (IFNγ). Tumor-specific T_H1 cells also control neoplastic lesion by favoring the activation of dendritic cells (DCs), natural killer (NK) cells, and M1 macrophages.[1] Therefore, the identification of peptides that activate both tumor-specific T_H1 cells and CTLs is an important goal for the induction of effective antitumor immune responses. Of note, DCs can process extracellular antigens and cross-present them on HLA class I molecules, leading to the activation of TAA-specific CD8+ T-cell responses. Such T-cell responses are considered to be crucial for both tumor eradiation and the generation of long-lasting immunological memory. Recent studies have shown that the administration of long peptides (LPs) encompassing T_H1 and CTL epitopes has the potential to elicit combined CD4+ and CD8+ T-cell responses through cross-presentation. Thus, anticancer vaccines based on a single polypeptide encompassing epitopes that elicit both T_H1 and CTL responses may exhibit superior efficacy as compared with peptides that activate either CD4+ or CD8+ T cells only.[2]

Nakamura et al. analyzed the gene expression profile of various cancers and normal tissues using a genome-wide cDNA microarray encompassing 27,648 genes. By this technology, we have identified a number of TAAs that are not expressed by normal adult tissues. Most often, the expression level of these TAAs was correlated with poor disease outcome. In particular, we have identified several HLA-A24*(A*24:02)*- or HLA-A2*(A*02:01)*-restricted CTL epitopes that are able to elicit tumor-reactive CTLs but not autoimmune responses.[3-5] In Phase I/II clinical trials involving lung, head and neck, pancreatic, gastric, and esophageal cancer patients, CTL epitopes derived from these novel TAAs were found to be safe and to induce not only robust TAA-specific CTL responses, but also a survival benefit, among individuals bearing advanced neoplasms.[6]

Based on these results, in order to further improve the efficacy of peptide-based anticancer immunotherapies, we attempted to identify TAA-derived long peptides (LPs) that can induce both TAA-specific T_H1 cells and CTLs (**Fig. 1**). To select a candidate LP bearing both T_H1 and CTL epitopes, we combined information from a recently developed computer algorithm predicting HLA class II-binding peptides with known HLA-A2(*A*02:01*)- or HLA-A24(*A*24:02*)-restricted CTL epitopes.[7,8] It is well known that the incidence of HLA-A2 and HLA-A24 is relatively high in various ethnic groups, including Asians, Africans, Afro-Americans, and Caucasians. In terms of TAAs, we focused on a cancer testis antigen, NDC80 kinetochore complex component (NUF2, best known as cell division cycle associated 1, CDCA1), and kinesin family member 20A (KIF20A).

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Submitted: 07/16/13; Accepted: 07/18/13
Citation: Tomita Y, Nishimura Y. Long peptide-based cancer immunotherapy targeting tumor antigen-specific CD4+ and CD8+ T cells. OncoImmunology 2013; 2:e25801; http://dx.doi.org/10.4161/onci.25801

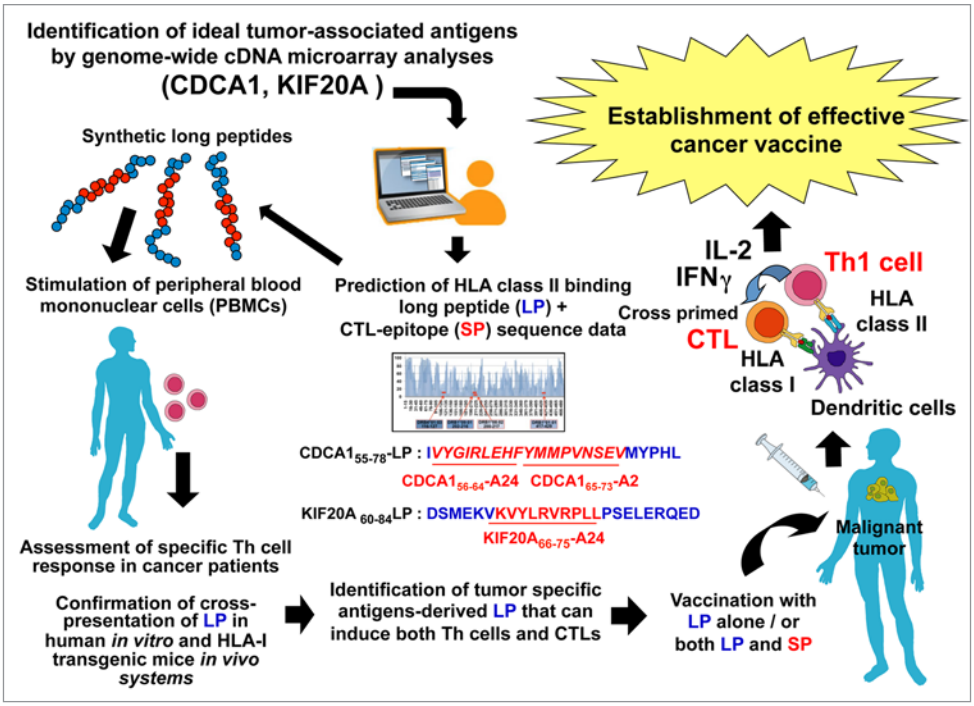

Figure 1. Identification of immunogenic long peptides encompassing both T_H1 and cytotoxic T lymphocyte epitopes. To select candidate CDCA1- and KIF20A-derived long peptides (LPs) that would encompass both T_H1 and cytotoxic T lymphocyte (CTL) epitopes, we combined the software-assisted prediction of HLA class II-binding peptides with known HLA-A2 or HLA-A24-restricted short CTL epitopes (SPs). Peripheral blood mononuclear cells (PBMCs) derived from healthy donors and cancer patients were used to investigate the immunogenicity as well as the in vitro cross-priming potential of these LPs. HLA-A2 or -A24 transgenic mice were employed to confirm the cross-priming potential of CDCA1- and KIF20A-derived LPs in vivo. LPs similar to those that we identified might allow for the propagation of both T_H1 and CTL responses in the course of cancer immunotherapy. IFNγ, interferon γ; IL-2, interleukin-2.

Thus, we succeeded in identifying highly immunogenic CDCA1- and KIF20A-derived LPs encompassing both T_H1 and CTL epitopes. Such LPs (24–26mers) encompassed naturally processed T_H1 epitopes that are presented by various HLA class II molecules including HLA-DR53, DR4, DR8, DR9, DR15, and DP2. Based on the findings, CDCA1- and KIF20A-derived LPs should be useful in at least 86% and 93% of the Japanese population, respectively.[7,8]

CDCA1- and KIF20A-derived LPs elicited T_H1 cells producing significant levels of IFNγ, intereleukin (IL)-2, tumor necrosis factor α (TNFα), granulocyte macrophage colony-stimulating factor (GM-CSF), and chemokine (C-C motif) ligand 4 (CCL4, also known as macrophage inflammatory protein-1β, MIP-1β), but comparatively lower amounts of IL-4, IL-6 and IL-17. The cytotoxicity marker CD107a was also detected on CDCA1- and KIF20A-specific T_H1 cells elicited by LPs. Moreover, we demonstrated that the cross-presentation of LPs efficiently primes tumor-specific $CD8^+$ CTLs, in vitro (with human cells) as well as in vivo (in *HLA-A2 (HHD)* or *A24 (HHH)* transgenic mice). Furthermore, the CDCA1-derived LP was superior to the corresponding (embedded) CTL epitope in the induction of CTL responses in vivo.[7,8]

We also assessed T_H1 responses specific for CDCA1- and KIF20A-derived LPs in the peripheral blood of cancer patients who were enrolled in phase I/II clinical trials based on three HLA-A24-binding, cancer testis antigen-derived CTL epitopes, namely, $CDCA1_{56-64}$-A24 (from CDCA1), $IMP\text{-}3_{508-516}$-A24 (from the U3 small nucleolar ribonucleoprotein protein, IMP3), and $LY6K_{177-186}$-A24 (from lymphocyte antigen 6 complex, locus K, LY6K). We indeed detected CDCA1- and KIF20A-specific T_H1 responses upon vaccination with CTL epitopes. Interestingly, the frequency of these responses was significantly higher in vaccinated cancer patients than in patients before vaccination and in healthy individuals. In addition, in some patients, antigen-specific T_H1 responses were boosted by the repeated administration of CTL epitopes. From these results, we speculate that tumor-specific T_H1 responses in vaccinated patients may result from phenomena of intramolecular epitope spreading or antigen spreading initiated by CTL epitopes.[7,8]

Recent clinical studies have demonstrated that targeting helper T cells and CTLs by the concomitant administration of HLA class I- and II-restricted epitopes enhance vaccine-dependent immune responses and improve clinical responses.[9] We investigated the synergistic effects of CDCA1- and KIF20A-derived LPs on the induction of antigen-specific CTLs by CTL epitopes. Both these LPs boosted the ability of CTL epitopes to elicit $tetramer^+$ and $CD107a^+$ CTLs. These findings indicate that LPs may be able to synergize with CTL epitopes in the elicitation of antigen-specific $CD8^+$ T-cell responses, hence exacerbating the clinical potential of this immunotherapeutic approach. The synergistic effects of anticancer vaccines and strategies aimed at blocking

immunological checkpoints, for instance antibodies targeting cytotoxic T lymphocyte-associated protein 4 (CTLA4), programmed cell death 1 (PD-1) or its ligand (CD274, also known as PD-L1), have previously been illustrated.[10] Thus, immunological checkpoint-blocking agents might constitute valid candidates for combination therapies involving CDCA1 and KIF20A-derived LP-based anticancer vaccines.

In conclusion, CDCA1- and KIF20A-derived LPs provide a good tool for the propagation of tumor-specific T_H1 cells and CTLs. Our studies support the clinical application of LP-based anticancer immunotherapy.

Disclosure of Potential Conflicts of Interest

Yasuharu Nishimura is supported by a funding from OncoTherapy Science, Inc.

References

1. Dobrzanski MJ. Expanding roles for CD4 T cells and their subpopulations in tumor immunity and therapy. Frontiers in oncology 2013; 3:63. PMID:23533029; http://dx.doi.org/10.3389/fonc.2013.00063.
2. Melief CJ, van der Burg SH. Immunotherapy of established (pre)malignant disease by synthetic long peptide vaccines. Nat Rev Cancer 2008; 8:351-60; PMID:18418403; http://dx.doi.org/10.1038/nrc2373
3. Imai K, Hirata S, Irie A, Senju S, Ikuta Y, Yokomine K, et al. Identification of HLA-A2-restricted CTL epitopes of a novel tumour-associated antigen, KIF20A, overexpressed in pancreatic cancer. Br J Cancer 2011; 104:300-7; PMID:21179034; http://dx.doi.org/10.1038/sj.bjc.6606052
4. Imai K, Hirata S, Irie A, Senju S, Ikuta Y, Yokomine K, et al. Identification of a novel tumor-associated antigen, cadherin 3/P-cadherin, as a possible target for immunotherapy of pancreatic, gastric, and colorectal cancers. Clin Cancer Res 2008; 14:6487-95; PMID:18927288; http://dx.doi.org/10.1158/1078-0432.CCR-08-1086
5. Harao M, Hirata S, Irie A, Senju S, Nakatsura T, Komori H, et al. HLA-A2-restricted CTL epitopes of a novel lung cancer-associated cancer testis antigen, cell division cycle associated 1, can induce tumor-reactive CTL. Int J Cancer 2008; 123:2616-25; PMID:18770861; http://dx.doi.org/10.1002/ijc.23823
6. Kono K, Iinuma H, Akutsu Y, Tanaka H, Hayashi N, Uchikado Y, et al. Multicenter, phase II clinical trial of cancer vaccination for advanced esophageal cancer with three peptides derived from novel cancer-testis antigens. J Transl Med 2012; 10:141; PMID:22776426; http://dx.doi.org/10.1186/1479-5876-10-141
7. Tomita Y, Yuno A, Tsukamoto H, Senju S, Kuroda Y, Hirayama M, et al. Identification of promiscuous KIF20A long peptides bearing both $CD4^+$ and $CD8^+$ T-cell epitopes: KIF20A-specific $CD4^+$ T-cell immunity in patients with malignant tumor. [Epub ahead of print.]. Clin Cancer Res 2013; In press; PMID:23714729; http://dx.doi.org/10.1158/1078-0432.CCR-13-0197
8. Tomita Y, Yuno A, Tsukamoto H, Senju S, Yoshimura S, Osawa R, et al. Identification of CDCA1 long peptides bearing both $CD4^+$ and $CD8^+$ T-cell epitopes: CDCA1-specific $CD4^+$ T-cell immunity in cancer patients. Int J Cancer 2013 *in press* PMID: http://dx.doi.org/10.1002/ijc.28376
9. Aarntzen EH, De Vries IJ, Lesterhuis WJ, Schuurhuis D, Jacobs JF, Bol K, et al. Targeting CD4(+) T-helper cells improves the induction of antitumor responses in dendritic cell-based vaccination. Cancer Res 2013; 73:19-29; PMID:23087058; http://dx.doi.org/10.1158/0008-5472.CAN-12-1127
10. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012; 12:252-64; PMID:22437870; http://dx.doi.org/10.1038/nrc3239