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Immunological validation of the EpitOptimizer program for streamlined design of heteroclitic epitopes

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Abstract

One strategy to generate T-cell responses to tumors is to alter subdominant epitopes through substitution of amino acids that are optimal anchors for specific MHC molecules, termed heteroclitic epitopes. This approach is manually error-prone and time-consuming. In here, we describe a computer-based algorithm (EpitOptimizer) for the streamlined design of heteroclitic epitopes. Analysis of two cancer-related proteins showed that EpitOptimizer-generated peptides have enhanced MHC-I binding compared with their wild-type counterparts; and were able to induce stronger CD8+ T-cell responses against their native epitope. These data demonstrate that this approach can serve as the basis of epitope-engineering against cancer and intracellular pathogens.
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1. Introduction

Development of cancer vaccines faces the fundamental difficulty that cancer arises from the host's tissue. Most commonly, cancer antigens are products of unaltered self-genes that are typically incapable of inducing primary immune responses. These self-proteins may contain epitopes with high affinity for self MHC-I molecules, but immature T cells with high affinity receptors for these self-peptides usually die during thymic development, and those that escape thymic negative selection are anergized or deleted in the periphery [1]. However, T cells with receptors with low or intermediate affinity for self-peptide/MHC survive thymic selection [1]. These subliminal peptide epitopes are unable to elicit T-cell activation and differentiation. Several groups, including ours, have studied strategies for generating effective immunity against self-antigens expressed by cancer. One such strategy is to design heteroclitic peptides that trigger recognition of subliminal epitopes presented by MHC-I molecule on the surface of tumor cells [1–7]. Using this approach, it is possible to alter the magnitude and specificity of T-cell responses by selectively changing (optimizing) amino acid residues involved in binding to MHC-I molecules. These optimized epitope variants can prime a subset of naïve cognate CD8+ T cells to cross-react against corresponding wild-type peptide/MHC complexes.

Computational algorithms to predict MHC binding of peptides were pioneered by the groups of Parker and Ram-

Abbreviations: MHC-I, major histocompatibility complex I; TYRP1, tyrosinase-related protein 1; PSMA, human prostate-specific membrane antigen; PSMAee, epitope enriched PSMA; APCs, antigen presenting cells

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mensee and have been routinely used to predict tumor-derived peptides that bind MHC-I and II molecules [8–10]. These algorithms are based on the isolation and amino acid sequences of naturally processed and presented MHC-I ligands and the MHC binding properties of synthetic peptides. The predictive capability of these computational algorithms comes from the identification of primary and secondary MHC-binding anchor residues. The binding score is predicted using quantitative matrices in which every amino acid in a peptide is assigned a coefficient. The overall binding value is calculated from amino acid coefficients. These algorithms have significantly facilitated the identification of tumor-derived peptides that bind MHC-I and II molecules, increasing the epitope arsenal of vaccine developers. Although EpitOptimizer builds on and extends these algorithms, the innovative aspect of this computer-based approach is that, it is designed to create multiple potential heteroclitic peptides with enhanced MHC-I binding along the entire length of polypeptides to induce greater immunogenicity.

Current efforts in cancer vaccine development have mainly focused on inducing CD8+ T-cell responses using native or heteroclitic epitopes [11–19]. Recently, we demonstrated that a non-immunogenic mouse self-protein is converted into an effective immunogen by an epitope optimization approach [7]. We manually designed multiple epitopes with improved binding to MHC-I molecules in mouse tyrosinase-related protein 1 (TYRP1) [30] (using site-directed mutagenesis). This approach combined MHC-I epitope optimization (creating heteroclitic epitopes), epitope enrichment (multiple epitopes), and enhancement of antigen processing through destabilization by mutations. DNA immunization with the engineered TYRP1 vaccine led to rejection of melanoma and significantly increased survival. The main caveat for these previous studies was the time-consuming and tedious analysis of the full-length protein and the laborious manual design of heteroclitic epitopes. EpitOptimizer was created to overcome these obstacles.

Here, we present EpitOptimizer and describe initial validation studies using a mouse melanoma differentiation antigen TYRP1, human prostate-specific membrane antigen (PSMA) [31] and five other self-molecules. Using this approach, we have discovered three novel HLA-A*0201-restricted human PSMA epitopes that are candidates for further evaluation in clinical studies. These data illustrate the capabilities of EpitOptimizer for selective epitope-engineering of poorly and non-immunogenic antigens.

2. Materials and methods

2.1. Mice

Female C57BL/6 mice were purchased from the National Cancer Institute breeding program (Frederick, MD). HLA-A*0201/K^b transgenic mice were obtained from Dr. Linda Sherman, The Scripps Research Institute (La Jolla, CA). All mice entered the study between 8 and 10 weeks of age. Care of all mice used in experiments was under an approved protocol and in accordance with the guidelines of the Institutional Animal Care and Use Committee of MSKCC.

2.2. Peptides and peptide vaccination

The peptides used in this study were synthesized crude with >80% purity by GeneMed Synthesis, Inc. (San Francisco, CA). They were resuspended in 100% DMSO at a concentration of 40 mg/ml, then diluted with PBS (final concentration 2 mg/ml) and frozen in aliquots at −20 °C. For vaccination, peptides were used at 1:1 with Titermax adjuvant (Sigma–Aldrich, St. Louis, MO). Recipient mice were vaccinated in the footpad with 20 μg peptide:adjuvant mixture. Five days after vaccination, mice were euthanized and draining inguinal lymph nodes harvested to assess *ex vivo* T-cell responses against wild-type peptides by ELIspot assay.

2.3. Antibodies and cell lines

Cell surface expression of D^b and HLA-A*0201 was assessed by staining with FITC-conjugated mAb anti-D^b mAb 28-14-8 or mAb A2.1-specific mAb BB7.2 or a FITC-conjugated isotype control Ab (BD Biosciences Pharmingen, San Jose, CA). The EL4 cell line is a C57BL/6 mouse lymphoma. RMA-S is a mouse transporter antigen peptide-deficient (TAP2) cell line [32]. K562/HLA-A*0201 is a human leukemia cell line transfected with HLA-A*0201 [37]. Human T2 cells are deficient in the transporter antigen peptide molecules (TAP1 and 2) [33].

2.4. MHC stabilization assay

To determine the relative ability of heteroclitic peptides to bind to MHC class I molecules, synthetic peptides corresponding to the wild-type and optimized predicted heteroclitic variants were tested by MHC class I stabilization assay [32]. Briefly, $\sim 2 \times 10^5$ cells TAP2-deficient K^b/D^b RMA-S cells were incubated overnight at 23 °C. Peptides were added at 1 μg/ml final concentration, followed by incubation for 2 h at 37 °C. TAP2-deficient human HLA-A*0201 T2 cells [32] were incubated with and without peptides for 16 h at 37 °C. Cells were then washed, and surface levels of D^b and HLA-A*0201 were assessed by staining with FITC-conjugated, mAb-D^b mAb 28-14-8 or mAb A2.1-specific mAb BB7.2 or an FITC-conjugated isotype control Ab (BD Biosciences Pharmingen, San Jose, CA). Cells were fixed at 4 °C in 4% paraformaldehyde and analyzed on a FACSCalibur (Becton Dickinson, Mountain View, CA) using CellQuest software. Results are expressed as MFI values.