Tumor Associated Antigens Harbor Readily Defined and Universally Immunogenic Regions Relevant for Cancer Immunotherapy

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ABSTRACT

Recent advances in cancer immunology, highlighted by immune checkpoint inhibitors, have demonstrated that immunotherapy is a viable option in the oncologist’s armamentarium. Despite these advances, many patients are non-responders; and strategies to convert non-responders into responders by inducing anti-tumor immune responses are needed. We hypothesized that many tumor associated antigens (Ag) are readily susceptible to immune attack, but only in the context of identifying the tumor antigen epitopes that can reliably initiate a response, regardless of individual patient human leukocyte antigen (HLA)-haplotypes. Utilizing known HLA-serotype frequencies and the open source epitope prediction algorithm netMHCIIPan, we made epitope predictions relevant for over ninety percent of the population from the widely expressed oncoproteins MUC1, HER2/neu and CMV pp65. Unexpectedly, predicted promiscuous HLA-binding epitopes clustered together in high priority regions: unique “hot spots” with high densities of non-HLA-serotype constrained epitopes. Synthetic long peptides spanning these regions were synthesized and utilized in an antigen specific T-cell culture system involving peripheral blood mononuclear cells (PBMC). Exposure of PBMC to these peptides was synchronized with aggressive surrogate activation of innate immunity by GM-CSF, LPS, and R848, followed by step 2 IL-7-modulated T-cell hyperegexpansion. Added long peptides (>20aa) derived from the “hot spot” regions of MUC1, HER2/neu, and CMVpp65 reliably produced selective and sustained expansion of both CD4+ and CD8+ peptide-specific, interferon-γ (IFNγ)-producing T-cells. MUC1 peptide-specific T-cells preferentially recognized MUC1 expressing tumor cell lines, either in tumor cell killing assays or as tumor lysate when pulsed onto restimulatory PBMC. This mechanistically rational antigen selection sequence, effective even for uncommon antigens, regardless of HLA-haplotypes, enables rapid identification of tumor protein regions relevant for cancer immunology, including adoptive immunotherapy, cancer vaccines, and even identification of tumor neo-antigens unique to each patient.

RESULTS

15mers Within “Hot Spot” Derived Peptides are Predicted to Bind to HLA-Serotypes More Strongly than 15mers Within Non-Hot Spot Derived Peptides: Each data point represents the average predicted IC50 for each 15mer, calculated from the predicted affinities for every HLA DR1 subtype examined. Lower values indicate higher affinity (two-tailed p<0.0001 for each comparison, applying Mann-Whitney test).

SEAU and SEA2 Driven T-cells Preferentially lyse MUC1 Expressing Tumor Targets: HLA-A2.1+ PBMC from 3 healthy donors were either driven polyclonally with anti-CD3/CD28 or driven by long peptides derived from the sea urchin sperm protein, enterokinase and sengin (SEA) domain of MUC1 (SEA1 and SEA2). At the end of T-cell culture expansion, each of the T-cell groups was cultured with PHA in a 1:100 ratio with C57-labelled HLA-A2.1+ human breast cancer line MDA-MB-231, either transduced to express MUC1 (MDA-MB-231.MUC1) or neo control (MDA-MB-231.Neo). % lysis was calculated as (Experimental lysis - Spontaneous C51 release)/Complete lysis in Triton X-100 - Spontaneous C51 release) x 100. % lysis of MDA-MB-231.Neo was statistically indistinguishable for all 3 donors whether cultures were polyclonally, SEA1 or SEA2 driven. SEA1 and SEA2 driven T-cells from all 3 donors lysed MDA-MB-231.MUC1 targets significantly more than MDA-MB-231.Neo targets (two-tailed SEA1 p=0.039 and SEA2 0.038, applying Student’s paired t-test).

CONCLUSIONS

- Tumor associated antigens harbor universally immunogenic regions which are readily defined and are attractive targets for cancer immunotherapy
- Tumor associated proteins harbor clusters of high priority areas that can speed the discovery of immunologic regions and screen out low priority domains unlikely to be relevant to cancer immunotherapy (Figures 2,4,6)
- CD4+ and CD8+ T-cells are easily expanded and enriched for tumor-Ag epitopes from high priority regions with the potential to recognize tumor derived Ag in multiple contexts (Figures 5,6,7)
- These findings are applicable to cancer vaccine development, adoptive immunotherapy, and immunotherapeutic strategies targeting patient specific neo-Ag

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